PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07K 14/205, A61K 39/106, C07K 16/12 | A1

(11) International Publication Number: WO 97/47646

(43) International Publication Date: 18 December 1997 (18.12.97)

(21) International Application Number: PCT/SE97/01009

(22) International Filing Date: 10 June 1997 (10.06.97)

(30) Priority Data:
9602287-6 10 June 1996 (10.06.96) SE
9701014-4 19 March 1997 (19.03.97) SE

(71)(72) Applicant and Inventor: BORÉN, Thomas [SE/SE]; Törelvägen 68, S-906 28 Umeå (SE).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): ARNQVIST, Anna [SE/SE]; Ö. Brinkvägen 55, S-903 21 Umeå (SE). NORMARK, Staffan [SE/SE]; Valhallavägen 126, S-114 41 Stockholm (SE). ILVER, Dag [SE/SE]; Bankgatan 18, S-902 35 Umeå (SE). HAMMARSTRÖM, Lennart [SE/SE]; Avd. för Klinisk Immunologi, Huddinge Sjukhus, S-141 86 Huddinge (SE).

(74) Agents: ONN, Thorsten et al.; AB Stockholms Patentbyrå, Zacco & Bruhn (publ), P.O. Box 23101, S-104 35 Stockholm (SE).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: HELICOBACTER PYLORI ADHESIN BINDING GROUP ANTIGEN

(57) Abstract

A novel *Helicobacter pylori* blood group antigen binding (BAB) adhesin protein was isolated and purified, whereby said protein or fractions thereof bind specifically to fucosylated blood group antigens. The protein sequence of said adhesin is disclosed in this application. Simultaneously the DNA sequences for two genes, babA and babB, producing highly similar proteins, are disclosed. Said adhesin and/or DNA is useful for diagnose and therapy and/or prophylaxis directed against *H. pylori* induced infections, e.g. gastritis and acid peptic disease, i.e. active vaccination. A new immunoglobulin composition, which exhibits specific activity to a Lewis^b antigen binding *Helicobacter pylori* adhesin, or preferably, monoclonal and/or polyclonal antibodies to said adhesin offer a new and more efficient method of treatment and/or prevention of gastrointestinal diseases, caused by *Helicobacter pylori* or other *Helicobacter* species, i.e. passive vaccination.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

HELICOBACTER PYLORI ADHESIN BINDING GROUP ANTIGEN

Field of the invention

The present invention relates to materials and methods for prevention, treatment and diagnosing of infections caused by *Helicobacter pylori*. More specifically the present invention relates to polypeptides and antibodies useful in vaccines for the treatment and prevention of pathologic infections caused by *Helicobacter pylori* strains. The present invention specifically relates to a bacterial blood group antigen binding adhesin (BAB-adhesin). The present invention further relates to polynucleotides useful for the recombinant production of said polypeptides and for use in immunisation therapies. In addition, it relates to polypeptides, antibodies, and polynucleotides used for the detection of said bacteria.

The present invention further relates to new immunoglobulins, which exhibit specific activity to a blood group binding adhesin, expressed by *Helicobacter pylori*, methods for the production of said immunoglobulins, their isolation and use. The present invention further relates to the treatment and prevention of *H. pylori* induced infections in the gastrointestinal tract.

Background of the invention

Helicobacter pylori is a causative agent for acid peptic disease and the presence of this organism is highly correlated to the development of gastric adenocarcinoma. Bacterial adherence to the human gastric epithelial lining was recently shown to be mediated by fucosylated blood group antigens.

Recent research has focused on the role of *Helicobacter pylori* in the development of ulcers in the gastric mucosa. Recent findings show a strong connection between *H. pylori* and chronic, active gastritis and gastric ulcers. Furthermore, there appears to be a strong correlation between ventricular cancer and gastric ulcers. Traditional treatment of gastric ulcers has involved gastric resection, the administration of bismuth compositions, the administration of H_2-blockers and the administration of pH-buffering agents, to mention a few examples.

More recently, various forms of treatment have been supplemented with the administration of antibiotics. One problem with presently known treatments is the risk for treatment failure. Furthermore, not only do microbes develop antibiotic resistance, the antibiotics administered often upset the natural balance of benign microbes, colonising the intestinal tract. This leads to diarrhoea and other signs of intestinal discomfort, in addition to destabilising the benign flora in the intestines. Other treatments, e. g. H_2-blockers often require life-long medication to prevent the recurrence of disease.

The foregoing, together with the fact that *H. pylori* is very widely spread among humans - according to a conservative estimate approximately 60 % of all adult humans in the industrialised countries are infected - makes the diagnosing, prevention and treatment of *H. pylori* infections an urgent task.

Further, the fact that developing countries frequently lack the resources for conventional treatment of gastric ulcers further underlines the importance of finding new ways of treatment and prevention of *H. pylori* induced infections. It is obvious, for many reasons, that disease prevention with vaccines is a preferable mode. A vaccine would provide an easily administered and economical prophylactic regimen against *H. pylori* infections. An effective vaccine against *H. pylori* is nevertheless presently lacking.

State of the art

H. pylori colonises the human gastric mucosa, in an equilibrium between adherence to the epithelial surface mucous cells and the mucous layer lining the gastric epithelium. Once infected, bacteria seems to colonise for a lifetime. Attachment to the epithelial lining protects the bacteria from the anti-microbial effects of the acidic gastric juice of the stomach lumen, as well as from physical forces such as peristalsis. For survival in this hostile ecological niche, *H. pylori* has developed a battery of virulence factors; such as production of the enzyme urease that buffers the micro-environment around the bacteria and the polar flagellae to ensure high motility, a prerequisite in an ecological niche where the turnover of the mucous layer is in the range of hours. A subset of *H. pylori* strains produces the vacuolating cytotoxin, VacA, and the cytotoxin associated antigen CagA .

Attachment is essential for colonisation of the epithelial lining and bacteria express surface associated adhesion molecules that recognise specific carbohydrate or protein receptors on the cell surfaces or mucous lining. The specificity in this interaction in combination with the genetically regulated receptor distribution results in a restricted range of cell lineages and tissues available for colonisation. Several putative receptor structures have been described for *H. pylori*, such as the hemagglutinin-sialic acid, sulphated glycoconjugates and sulphatides. Recently, the fucosylated blood group antigens H-1 and Lewis^b were described (Borén *et al.*, Science, 262, 18921993), mediating specific adherence of *H. pylori* to human and rhesus monkey gastric surface mucous cells *in situ*. The H-1 and Lewis^b antigens are part of the blood group antigens that define blood group O in the ABO system.

Surface-exposed proteins are often constituents of the outer membrane. The outer membrane has a structural role and acts as a selective barrier, determining what enters the cell

and what molecules are secreted. One class of outer membrane proteins are called porins, and create hydrophilic pores through the outer membrane where specific metabolites, such as sugar molecules, can cross. Recently the finding of a number of outer membrane proteins in *H. pylori*, was reported, which proteins were suggested to constitute a family of porin proteins.

The BAB adhesin has previously been identified and shown to be localised on the bacterial surface of *H. pylori* (SE 9602287-6). The blood group binding activity was shown to be pH dependent and the present inventors present evidence that the binding affinity to the Lewis^b receptor reveals a high equilibrium constant. For the purification of the BAB adhesin, a crosslinker-labelled receptor conjugate was used in order to mediate specific transfer of biotin to the adhesins on the bacterial surface. Thereafter the biotin-labelled adhesin could be extracted by streptavidin coated magnetic beads. Determination of the amino terminal amino acid sequence of the purified BAB adhesin exhibit homologies to outer membrane proteins of *H. pylori* porins.

Intensive research has been directed to the immunological treatment and prevention of *H. pylori* induced infections. EP 0 484 148 (Ando & Nakamura) describes a method for treating and/or preventing upper gastrointestinal disease in mammals, said method comprising orally administering to a patient in need thereof an effective amount of a pharmaceutical composition comprising anti-*Helicobacter pylori* polyclonal immunoglobulins and a pharmaceutically acceptable carrier. Said description further dwells on the combination of said treatment in combination with the administration of antibiotics. As the method of producing said polyclonal antibodies, EP 0 484 148 describes the isolation and purification of anti-*H. pylori* immunoglobulins from the sera and milk of mammals. *H. pylori* itself was not found in the stomachs of cows, goats, sheep, swine or horses, according to EP 0 484 148, but it was assumed that these animal species have colonizing microorganisms with antigenic determinants similar to those of *H. pylori* because they have immunoglobulins which cross-react to strains of *H. pylori* found in humans. Preferably, according to EP 0 484 148, large mammals, e.g. pregnant cows, are immunized with whole cells of *H. pylori* and the immunoglobulins subsequently extracted from the milk or colostrum. In the immunization experiments, NCTC Strain 11362 and clinical isolate *H. pylori* No. 153 were used to trigger the production of immunoglobulins. On the other hand, NCTC Strain 11637 was used for analysing purposes. Immunization is claimed to yield an anti-*H. pylori* titer in the milk of such magnitude, that daily doses of 0.01-0.1 g/day immunoglobulin composition, are sufficient for successful therapy. The claimed interval of

0.01-0.1 g/day is however not supported by the experiments presented by Ando & Nakamura and so low doses have hitherto not proven efficient in clinical tests. The doses actually used in example 5 and 7 are in the order of magnitude of 1 g/day, i.e. 10-fold the upper limit of the given interval. Furthermore, it is very unlikely, that unspecific immunoglobulin mixtures as those manufactured by Ando & Nakamura, would be effective in claimed doses as similar doses are ineffective against other gastrointestinal pathogens. The simultaneous administration of antibiotics, extensively discussed in the description, underlines the insufficiency of the disclosed immunoglobulins.

EP 0 469 359 (Cordle & Schaller) likewise describes the immunization of mammals, preferably pregnant cows, with formalin killed *H. pylori* bacteria (ATCC Strain 26695). Anti-*H. pylori* polyclonal antibodies were isolated and purified from the milk and finally fed to piglets, in amounts of about 0.5 g immunoglobulins, three times daily. The results were assessed by determination of the number of biopsy specimens, which were positive for Gram-negative bacteria after the trial. Gram-negative bacteria was found in 78 % of the piglets fed a non-immune nutrient but only (Sic!) in 35 % of the piglets fed a nutrient containing so called specific anti-*H. pylori* antibodies.

Anti-*H. pylori* polyclonal antibodies, effective to cause aggregation of *H. pylori*, have thus been administered orally as a regimen in the treatment and prevention of *H. pylori* induced infections in the gastrointestinal tract. Nevertheless, as also noted in EP 0 484 148 A1, it is still not clear, how many antigenic determinants are present on the surface of *H. pylori*. The occurrence of a wide variety of *H. pylori* strains, makes questionable the practical efficiency of any polyclonal immunological therapy according to the state of the art. Immunization using whole bacteria will always trigger a highly polyclonal immunresponse with a low level of antibodies against a given antigenic determinant. This is underlined e.g. by the results presented by Cordle & Schaller, where, although the number of *Helicobacter* positive biopsies were reduced, complete cure was not obtained through the treatment according to their invention.

It is notable, that the dose of immunoglobulin needed for oral prophylaxis or therapy has not yet been clearly defined. In a normal human adult, approximately 5 g IgA is produced and secreted at mucosal surfaces each day. Obviously, doses of this magnitude are economically and practically unfeasible for large-scale therapy or prophylaxis. In studies on the effect of oral immunoglobulin on rotavirus infection, daily doses in the interval of 600

to 9000 mg have been tried in clinical tests. Successful intervention has also been reported when treating H. pylori and cryptosporidial infections with daily administrations of 3 to 15 g immunoglobulin from immunized cows (Hammarström et al., Immunol Rev, 139 (1994) 43-70). Generally speaking, all studies hitherto point to the necessity of using high doses of immunoglobulins when trying to combat an ongoing infection. The need for more specific immunoglobuline preparations, allowing the use of smaller doses, is thus an urgent one.

To maximize the potency of an immunological regimen for the treatment and prevention of *H. pylori*, it is of great importance to find a specific conserved antigenic determinant, which plays a central role for the pathogenicity of *H. pylori*. Using such an antigenic determinant would make it possible to produce highly specific and therapeutically efficient novel polyclonal and/or monoclonal immunoglobulin preparations.

Summary of the invention

The above problem of providing specific, cost-efficient and therapeutically superior immunoglobulin preparations for the treatment and prevention of *H. pylori* has now been solved through the composition and methods according to the attached patent claims. The present inventors have now surprisingly shown, that highly specific and therapeutically efficient polyclonal and/or monoclonal immunoglobulin preparations can be provided through the immunization of an animal with an adhesin protein, specific for *H. pylori*. Said adhesin protein is characterized already in the priority applications SE 9602287-6 and SE 9701014-4, which hereby are referred to in their entirety. The invention will now be described in closer detail with reference to the attached, non-limiting figures and examples.

One objective of the present invention was to further purify and characterize the *H. pylori* blood group antigen binding (BAB) adhesin to make possible the development of methods and materials for specific and selective diagnosing and treatment of *H. pylori* induced infections and related diseases and the development of said methods and materials. A further and equally important objective was to determine the DNA sequences of the genes involved in the expression of this protein. These objectives were fulfilled through the protein specified in claim 1, the DNA disclosed in claim 13 and 14 and the methods and materials specified in the subsequent claims. The DNA sequences are attached as Appendix 1 and 2, disclosing the babA and babB sequences, respectively. The full protein sequence is disclosed in Appendix 3.

Description of the figures

Fig. 1 A) illustrates the bacterial binding to soluble blood group antigens. *H. pylori* strains were incubated with ^{125}I-labeled blood group antigen glycoconjugates and bound ^{125}I -activity

was measured (Note the absence of blood group antigen binding shown for strains MO19 and 26695.),

Fig. 1 B) illustrates an receptor displacement assay. Strain CCUG 17875 was first incubated with 10 ng ^{125}I-labeled Le^b antigen glycoconjugate and the complex was then challenged (1 h) with an excess of unlabeled Le^b or Le^a glycoconjugate, before the ^{125}I-activity in the bacterial pellet was measured. Concentrations of the unlabeled glycoconjugate ranged from 50 ng to 8 μg and C) shows the results of a Scatchard analysis of the *H. pylori*-Le^b antigen interaction. Bacterial binding to the Le^b glycoconjugate was titrated to an affinity constant (Ka) value of $8\text{x}10^{-10}$ M^{-1} (13).

Fig. 2: Upper panel: Prevalence of the BabA adhesin in the bacterial population. Cells of strain CCUG 17875 were incubated with biotinylated Le^b (A) or Le^b (B) glycoconjugate. Bound biotinylated Lewis-conjugate was detected with FITC-labeled streptavidin (green fluorescence) and bacteria were counterstained with propidium iodine (red fluorescence). Lower panel: Localisation of the BabA adhesin. For electron microscopy (15) cells of strain CCUG 17875 were incubated with biotinylated Le^b (C) or Le^a (D).

Fig. 3 shows the characterization of the molecular weight of the BabA adhesin by the use of receptor overlay analysis (A, B) and receptor activity directed affinity tagging of BabA (C).

Fig. 4 shows receptor activity directed affinity tagging and protein purification of the BabA adhesin.

Fig. 5 shows the translated amino acid sequences for the babA and babB genes, corresponding to the N-terminal domain of the BabA adhesin.

Fig. 6 shows the procentual inhibition of *H. pylori* binding to ^{125}I-labeled Lewis b antigen for different preparations as a function of the antibody titre.

Fig. 7 shows a Western blot detection of the BabA adhesin by the different antibody preparations.

Fig. 8 shows four Western blot analyses of *H. pylori* proteins by the different antibody preparations.

Description of the invention

The blood group antigen binding adhesin, BabA, has now been biochemically characterized and purified by a novel technique, receptor Activity Directed Affinity Tagging (Retagging). Two genes, babA and babB were found to code for two different but very similar proteins. The present invention thus comprises a novel blood group antigen binding adhesin according to claim 1 and the subsequent claims. The DNA sequences are disclosed in appendices 1 (babA) and 2 (babB). The protein sequences is disclosed in appendix 3. The

invention also includes any pharmaceutical composition comprising said adhesin protein and/or fractions thereof. Examples of such pharmaceutical compositions are for example medicaments for the prevention or treatment of *Helicobacter pylori* induced gastritis, gastric and duodenal ulcers and gastric adenocarcinoma. Optionally said pharmaceutical composition additionally encompasses pharmaceutically acceptable excipients.

Further, the present invention comprises the BAB-adhesin gene or genes for expression of an adhesin protein according to the invention. Said invention also comprises a novel method for the isolation and purification of said adhesin. The disclosed genes are contemplated to function as a cassette system, the organism alternating between these to avoid immunity in the host. It is very likely, that homologies of the disclosed sequences exist and additionally supplement said cassette function in other strains of *H. pylori*. Also, genes corresponding to a homology of the first 40 amino acids or genes, corresponding to a homology of the last, about 300 amino acids, can function to this effect. It is further highly likely, that *Helicobacter pylori* is able to switch between several genes, similar to the disclosed genes, in a so-called cassette system.

The invention additionally comprises monospecific antisera produced using the novel adhesin protein and/or fractions thereof. Said monospecific antisera is preferably produced according to any suitable, conventional method for producing monospecific antisera *in vitro* or *in vivo*, e.g. by inoculating a suitable animal. Such methods are familiar to a person skilled in the art. Antibodies raised in a suitable animal or in the patient to be treated, can subsequently be administered locally, e.g. orally to the patient.

The invention further comprises the use of said monospecific antisera for the manufacturing of a test kit for quantitative or qualitative determinations of adhesin protein or fractions thereof in cells, tissues or body fluids.

The invention further comprises the use of said adhesin protein or corresponding DNA for use in therapy or immunisation and/or in the manufacture of compositions for said uses. The invention specifically encompasses the use of said DNA for immunisation therapy and for the manufacture for compositions for such therapy. Preferably, in an immunisation therapy where said composition is administered orally to a patient, the adhesin protein, fractions thereof or said DNA is administered in combination with a pharmaceutically suitable immunostimulating agent. Examples of such agents include, but are not limited to the following: cholera toxin and/or derivatives thereof, heat labile toxins, such as *E. coli* toxin and similar agents. The composition according to the present invention can further include conventional and pharmaceutically acceptable adjuvants, familiar to a person skilled in the art of

immunisation therapy. Preferably, in an immunisation therapy using the inventive DNA or fractions thereof, said DNA is preferably administered intramuscularly, whereby said DNA is incorporated in suitable plasmide carriers. An additional gene or genes encoding a suitable immunostimulating agent can preferably be incorporated in the same plasmide.

Said immunisation therapies are not restricted to the above-described routes of administration, but can naturally be adapted to any one of the following routes of administration: oral, nasal, subcutaneous and intramuscular. Especially the oral and nasal methods of administration are promising, in particular for large-scale immunisations.

The present inventors have surprisingly shown, that highly specific and therapeutically efficient polyclonal and/or monoclonal immunoglobulin preparations can be provided through the immunisation of an animal with an adhesin protein or fractions thereof, specific for *H. pylori*. When considering immunisation against *H. pylori*, it is worth noting that the infection is known to be lifelong despite a vigorous immune response in the gastric mucosa. An increased local production of IgA in the mucosa is not necessarily enough and the administration of monospecific antibodies directed against a central virulens factor, such as the adhesin according to the present invention, may constitute a more effective approach.

The term "immunisation" refers here to a method for inducing a continous high level of antibody and/or cellular immunresponse. The term "animal" here preferentially denotes any member of the subphylum Vertebrata, a division that includes all animals, including mammals, which are characterized by a segemented bony or cartilaginous spinal column. All vertebrates have a functional immune system and respond to antigens by producing antibodies. The term "protein" is used here to denote a naturally occurring polypeptide and the term "polypeptide" is used here in its widest meaning, i.e. any amino acid polymer (dipeptide or longer) linked through peptide bonds. Accordingly the term "polypeptide" comprises proteins, oligopeptides, protein fragments, analogues, muteins, fusion proteins and the like. The term "antibody" as used in this context includes an antibody belonging to any of the immunological classes, such as immunoglobulins A, D, E, G or M. Of particular interest are nevertheless immunoglobulin A (IgA) since this is the principle immunoglobulin produced by the secretory system of warm-blooded animals. However, in cow colostrum, the main antibody class is IgG 1.

Borén et al. have recently isolated and characterized a Lewis^b binding protein with a molecular weight of about 73500 Da (See the priority applications SE 9602287-6 and SE

9701014-4, which are referred to in their entirety). This adhesin protein is thought to be a conserved structure and specific for pathogenic strains of *H. pylori*. Said protein is specific for at least one of the *H. pylori* strains included in the following group: CCUG 17875, NCTC 11637, A5, P466, G109, G56, Ba 185, Ba 99, 931 and 932.

This adhesin protein or immunologically effective fractions thereof are characterized in that the following amino acid sequence is included:

EDDGFYTSVGYQIGEAAQMV

or homologues thereof.

The following DNA sequence or homologues thereof is included in DNA for expression of said adhesin protein or fractions thereof:

5´- GAAGACGACGGCTTTTACACAAGCGTAGGCTATCAAATCGGT
GAAGCCGCTCAAATGGTA - 3´

According to one embodiment of the invention, a pregnant mammal, preferably a cow or another suitable domestic animal, is immunised with said Lewis^b binding adhesin protein or fractions thereof. The adhesin protein or fractions thereof is/are preferably injected intramuscularly or subcutaneously in the chosen animal, optionally together with suitable adjuvants. Examples of such adjuvants include, but are not limited to immunostimulating agents such as cholera toxin and/or derivatives thereof, heat labile toxins, such as *E. coli* toxin and similar, conventional agents, such as classical adjuvants including mineral and vegetable oils. Subsequent to the regimen of immunization, comprising a necessary amount of doses, including so called booster-doses, over a time span allowing for optimal immunoglobulin expression, milk or sera is collected from said animal. Preferably the cow colostrum, which is specially high in immunoglobulins, is collected. The specific immunoglobulin fraction according to the present invention is then separated and purified in a conventional manner, e g including separation of fats, protein precipitation and concentration by ultrafiltration.

According to another embodiment of the invention, a bird, preferably a chicken or another suitable domestic bird, is immunized with said Lewis^b binding adhesin protein or fractions thereof. The adhesin protein or fractions thereof is preferably injected intramuscularly or subcutaneously in the chosen bird, optionally together with suitable adjuvants. Examples of such adjuvants include, but are not limited to immunostimulating agents such as cholera toxin and/or derivatives thereof, heat labile toxins, such as *E. coli* toxin and

similar, conventional agents, such as classical adjuvants including mineral and vegetable oils. Subsequent to the regimen of immunization, comprising a necessary amount of doses, including so called booster-doses, over a time span allowing for optimal immunoglobulin expression, sera or eggs is/are collected from said animal. Preferably the egg yolk, which is specially high in immunoglobulins, is collected. The specific immunoglobulin fraction according to the present invention is then separated and purified in a conventional manner, e g including protein precipitation and ultrafiltration. Alternatively, the egg yolk being of high nutritional value in addition to containing a high titer of specific antibodies according to the present invention, can be administered as such.

According to a preferred embodiment of the present invention, monoclonal immunoglobulin is produced by establishing transgenic animals. Said transgenic animals can be chosen from the following group of species: mammals, e.g. cow, goat and rabbit, and birds: e.g. chicken, duck, turkey. The mammal most preferably used is cow and the most preferable bird is chicken. Further developments of transgenic animals such as mice and rats could also offer new possibilities. The choice of animal is naturally governed by availability and local adaptation.

According to one embodiment, a stock of transgenic animals according to the present invention, adapted to the local conditions, are kept locally, e.g. in villages in developing countries to function as local units for the production of immunoglobulins for oral administration. For example transgenic cows, goats or chicken are suitable for this purpose and preferably chicken are used. Consumption of the milk or preferably the eggs, produced by the transgenic animals, can help to eradicate presently very difficult infectious diseases, e.g. diseases caused by *H. pylori*.

According to yet another embodiment of the present invention, monoclonal antibodies can be produced using the hybridoma method. The hybridoma method is well known to a skilled worker in the field of biochemistry and it is described e. g. in Galfre, G. And Milstein, C., Preparation of monoclonal antibodies: strategies and procedures (Methods in Enzymology, 73:3-46, 1981). A suitable host animal is immunized with the Lewis^b binding adhesin protein or fractions thereof. When the immunization is accomplished, the animal is sacrificed, spleen cells collected and fused with cells from a neoplastic cell line, preferably myeloma cells. By choosing the growth conditions, the successfully fused hybridoma cells

can be selected. The monoclonal antibodies produced by the hybridoma cell line can then be administered orally in a regimen for treatment and/or prevention of *H. pylori* infections.

Preferably the polyclonal and/or monoclonal antibodies are purified prior to administration and, more preferably, admixed with pharmaceutically suitable carriers and/or adjuvants. Examples of suitable carriers are saline, pharmaceutically acceptable fats, oils, carbohydrates and proteins. The carrier or carriers is/are preferably chosen so that the solubility and absorption of the immunoglobulin in the mucus layer lining the stomach is enhanced. Using suitable adjuvants the stability, therapeutic efficiency and nutritional value of the composition can be improved. To improve stability under storage, the immunoglobulin composition can be lyophilized. Regardless of the exact preparation and formulation, it is of central importance to avoid denaturating the immunoglobulins.

The higher specificity, exhibited by the immunoglobulin preparation of polyclonal and/or monoclonal antibodies according to the invention, makes it possible use substantially lower doses compared to those presently used, thus lowering the cost and improving the availability of the treatment. The use of specific, monoclonal antibodies can make it possible to further lower the doses. The doses are in all cases a function of the antibody titer of the preparation. A high titer naturally allows the use of lower doses.

According to one embodiment of the invention, an immunoglobulin preparation is manufactured as follows: an animal is immunized with a Lewis^b binding adhesin protein or fractions thereof, expressed by *Helicobacter pylori*, the immunoglobulin fraction is isolated from a excretion of said animal and subsequently purified. The purified immunoglobulin composition is admixed with suitable carriers and adjuvants to form a immunoglobulin preparation for the prevention or treatment of *H. pylori* infections. In cases where the antibody titer is sufficiently high and the other constituents of the immunoglobulin composition isolated from the animal are harmless, for example in the case of colostrum from immunized cows or egg yolk from immunized chicken, there is always the option of administering the colostrum or egg yolk to the patient without any further treatment of the colostrum or egg yolk.

The immunoglobulin composition according to the invention is preferably administered orally to the patient, in the smallest therapeutically or prophylactically effective dose. Presently conceived are doses in the interval of 0.1 to 1000 mg/day, preferably in the interval of 0.1 to 100 mg/day. The chosen doses naturally depend on the antibody titer of

the preparation in question. The exact doses and the regimen of administration can be chosen by the physician responsible for the patient, infected by *Helicobacter pylori*. Routine experimentation and later, with increasing experience of this method, empirical information will suffice to establish the required amount. Multiple dosages may be used, as needed, to provide the desired level of therapeutic or profylactic effect. The immunoglobulin preparations according to the present invention can also, being free from adverse side effects and imposing practically no danger of overdosing, be taken prophylactically or therapeutically by a person without medical supervision.

A therapeutical effect can be attained, except with the specific antibody according to the present invention, also with at least two Fab-fragments of said antibody. Said embodiment is also encompassed by the scope of the present invention.

According to yet another embodiment, avirulent microorganisms, preferably bacteria, are used as expression systems for the specific antibody according to the present invention. An "avirulent microorganism" in this context is a microorganism which has the ability to colonize and replicate in an infected individual, but which does not cause disease symptoms associated with virulent strains of the same species of microorganism. The definition inherent in the GRAS (Generally Regarded As Safe) concept can be applied here. A GRAS-organism is suitable for use according to the present invention, provided that the organism externalises the antibody or can be modified to this effect. The term "microorganism" as used herein includes bacteria, protozoa and unicellular fungi. Preferably, bacteria are used as expression systems, e.g. bacteria of the genus *Lactobacillus, Streptococcus* or *Enterobacteriae*. The above mentioned expression system can be utilised *in vitro* for the production of the specific antibody according to the present invention or, according to a further embodiment of the invention, the micro-organism constituting the expression system can be administered directly to the patient. The micro-organisms can be harvested and administered as such, but they are preferably mixed with a suitable carrier, mixed in a suitable foodstuff, lyophilised, encapsulated or treated in any other conventional way, used for the delivery of viable micro-organisms to the gastrointestinal tract.

According to yet another embodiment, avirulent microorganisms, preferably bacteria, are used as expression systems for the specific adhesin protein according to the present invention. An "avirulent microorganism" in this context is a microorganism which has the ability to colonize and replicate in an infected individual, but which does not cause disease

symptoms associated with virulent strains of the same species of microorganism. The definition inherent in the GRAS (Generally Regarded As Safe) concept can be applied here. A GRAS-organism is suitable for use according to the present invention, provided that the organism externalises the adhesin protein or can be modified to this effect. The term "microorganism" as used herein includes bacteria, protozoa and unicellular fungi. Preferably, bacteria are used as expression systems, e.g. bacteria of the genus *Lactobacillus, Streptococcus* or *Enterobacteriae*. The above mentioned expression system can be utilised *in vitro* for the production of the specific adhesin according to the present invention or, according to a further embodiment of the invention, the micro-organism constituting the expression system can be administered directly to the patient. The micro-organisms can be harvested and administered as such, but they are preferably mixed with a suitable carrier, mixed in a suitable foodstuff, lyophilised, encapsulated or treated in any other conventional way, used for the delivery of viable micro-organisms to the gastrointestinal tract.

The exact doses and the regimen of administration of said micro-organisms can be chosen by the physician responsible for the patient, infected by *Helicobacter pylori*. Routine experimentation and later, with increasing experience of this method, empirical information will suffice to establish the required amount. Multiple dosages may be used, as needed, to provide the desired level of therapeutic or prophylactic effect. The avirluent micro-organism expressing the antibody or adhesin protein according to the present invention can also, being free from adverse side effects and imposing practically no danger of overdosing, be taken prophylactically or therapeutically by a person without medical supervision. A preferred carrier in this specific application is a foodstuff, e.g. a fermented product such as fermented cereal or dairy product.

The creation of previously mentioned expression systems and still earlier mentioned methods of creating hybridomas and transgenic animals can include steps involving recombinant DNA techniques. Recombinant DNA techniques are now sufficiently well known and widespread so as to be considered routine. In very general and broad terms, recombinant DNA techniques consist of transferring part of the genetic material of one organism into a second organism, so that the transferred genetic material becomes a permanent part of the genetic material of the organism to which it is transferred. Methods for achieving this are well known and the mere choice of specific methods for achieving the

objectives, set out in the present description and claims, fall under the scope of the invention.

It is possible, that *H. pylori* alone or together with related slow-acting bacteria are involved in the genesis and aggravation of other chronic inflammatory diseases in the gastrointestinal tract. It is obvious for a skilled practitioner how to modify the present invention, within the scope of the claims, to gain utility in the treatment and/or prevention of such diseases. Examples of such diseases are ulcerative colitis, Crohn´s disease, sarcoidosis, Wegener´s granulomatosis and other vasculithic disorders, as well as various neoplasms, including carcinomas of the colon, pancreas and prostate.

Examples

H. pylori strain CCUG 17875 was obtained from CCUG, Göteborg, Sweden. Strain A5, a gastric ulcer isolate, from Astra Arcus, Södertälje, Sweden. Strains P466 and MO19 were described previously (Borén et. al, Science, 262, 1892(1993)). Strain 26695 came from Dr. K.A. Eaton, The Ohio State University and its genome was recently sequenced by TIGR, Rockville, Maryland, USA. The panel of 45 *H. pylori* clinical isolates came from the University Hospital in Uppsala, Sweden. Bacteria were grown at 37 °C in 10 % CO2 and 5 % O2 for 48 h.

All blood group antigen glycoconjugates used, i.e. semi-synthetic glycoproteins constructed by the conjugation of purified fucosylated oligosacharides to serum albumin were from IsoSep AB, Tullinge, Sweden. The RIA was performed according to Falk et al. (Meth. Enzymol., 236, 353, 1994) with some modifications; the H-1, Le^b, Le^a, H-2, Le^x and Le^y glycoconjugates were 125I-labeled by the Chloramine T method. 1 ml of bacteria (A600=OD 0.10) was incubated with 300 ng of 125I -labelled conjugate (i.e. an excess of receptors) for 30 min. in phosphate buffered saline (PBS), 0.5 % albumin, 0.05 % Tween-20 (BB-buffer). After centrifugation, 125I-activity in the bacterial pellet was measured by gamma scintillation counting.

In this study the present inventors' first biochemically characterized and identified the *H. pylori* <u>b</u>lood group <u>a</u>ntigen <u>b</u>inding <u>a</u>dhesin, BabA. *H. pylori* strains were analysed for binding to soluble ^{125}I-labeled fucosylated blood group antigens (Fig. 1A). Binding of these strains to the soluble blood group antigens correlate with adherence *in situ*. The prevalence of <u>b</u>lood group <u>a</u>ntigen <u>b</u>inding (BAB)-activity was assessed among 45 clinical *H. pylori* isolates and the majority of the isolates, 71%, express Le^b antigen binding properties (data not shown). In contrast, none of the reference strains (Fig. 1A), or strains from the panel of 45 clinical

isolates, bind to the Le^a, H-2, Le^x, or Le^y antigens. These results support our previous findings of high receptor specificity for the Le^b and H-1 blood group antigens and demonstrate the high prevalence of BAB activity among clinical isolates.

Based on the presence or absence of virulence factors such as the Cytotoxin associated gene A (CagA) and the Vacuolating cytotoxin A (VacA), *H. pylori* strains are classified as type I or type II strains. *H. pylori* isolates from patients with duodenal ulcers most often express the VacA and the CagA-proteins, i.e. type-I strains. By definition, type II strains express neither markers. Twenty-one clinical isolates previously defined for expression of CagA and VacA were analysed for Le^b antigen binding properties. Expression of CagA was found to correlate with bacterial binding to the Le^b antigen (Table 1). The *cagA* gene belongs to a 40 kb pathogenicity island that encodes components of secretion and transport systems. These findings could indicate functional crosstalk between the *cag* pathogenicity island and the BabA adhesin gene, for the correct presentation of the BabA adhesin protein in the bacterial outer membrane.

To further characterize BabA, the present inventors determined the affinity constant (K_a) between BabA and the Le^b antigen. Since K_a-values are based on equilibrium conditions (13), the present inventors first analysed the interaction by performing receptor displacement analysis. *H. pylori* CCUG 17875 (positive for Le^b binding, Fig. 1 A) was first incubated with ^{125}I-labeled Le^b glycoconjugate. Then unlabeled Le^b glycoconjugate was added in a dilution series. The unlabeled Le^b conjugate displaced the bound ^{125}I-labeled Le^b glycoconjugate efficiently (Fig. 1 B). The results demonstrate that the receptor-adhesin complex formed is in a true state of equilibrium. An equivalent excess of Le^a glycoconjugate did not dissociate the Le^b-BabA complex, verifying the high receptor specificity (Fig. 1B). The K_a-value for the Le^b-BabA complex of strain CCUG 17875 was titrated with Le^b glycoconjugate in concentrations from 10 ng to 260 ng/ml and determined to be of an high affinity close to $1 \times 10^{10} M^{-1}$ (Fig. 1C). The number of Le^b glycoconjugate molecules bound to BabA on the bacterial cell surface was calculated to be around 500 per cell. This number is similar to the number or fimbriae organelles on the surface of *E. coli* (14). However, for the BabA adhesin, the calculations are based on the assumption that the majority of bacterial cells in the experiment exhibit an equal number of adhesin molecules with Le^b antigen binding properties.

Table 1 BAB activity among H. pylori Type I and Type II strains

Typ	Strain	BAB activity
Type I	CCUG 17874	-
CagA+,VacA+	G39	-
	G11	-
	G20	-
	G27	+
	G56	+
	G106	-
	G109	+
	932	+
	Ba185	+
	87A300	+
Type Ia	931	+
CagA+,VacA-	Ba99	+
	Ba179	+
	Ba194	+
Type Ib	G12	-
CagA-,VacA+		
Type Id	G104	-
Δ*cagA*, VacA+	Tx30	-
Type II	G21	-
CagA-, VacA-	G50	-
	G198	-

To determine the prevalence of BabA in the bacterial population, strain CCUG 17875 was incubated with Le^b or Le^a antigens, and bacterial binding activity was visualised by confocal fluorescence microscopy (Fig. 2, upper panel). The analyses demonstrate the high prevalence of BabA binding activity in the bacterial population to the Le^b antigen (Fig. 2A, green staining) and the complete lack of binding to the Le^a antigen (Fig. 2B, red counter staining).

Next, the localisation and density of BabA on the bacterial cell surfaces was investigated by immunogold electron microscopy. The Le^b antigen binding activity of the adhesin localised gold particles to the bacterial outer membrane (Fig. 2C). Individual bacterial cells exhibit an equal number of gold particles (data not shown). When the Le^b antigen was substituted with the Le^a antigen (lacking receptor activity), no gold particles were detected (Fig. 2D).

The molecular weight of BabA was characterized by receptor overlay analysis. A protein extract of strain CCUG 17875 was separated on SDS-PAGE and blotted to a membrane. The membrane was incubated with biotinylated Le^b glycoconjugate, followed by detection with streptavidin and enhanced chemiluminescence. The BabA adhesin activity corresponds to a single 74 kDa band (Fig. 3A). The 40 kDa band is presumably endogenous peroxidase activity since it stains independently of the Le^b conjugate overlay (lane 3). BabA was very heat stable and could regain some activity after heating to 97°C (Fig. 3A; lane 2). The panel of strains exhibited the same molecular weight of BabA (Fig. 3B).

To purify BabA, a novel technique was developed, Receptor Activity Directed Affinity Tagging (ReTagging). Multi-functional crosslinking agents with radiolabeled donating tags have been previously used for receptor-ligand characterization studies. However, the use of affinity donating tags, such as biotin residues presented on flexible spacer structures, adds a new dimension to the applicability of crosslinker technology. An affinity tag, biotin, is transferred to the adhesin protein by the receptor activity and is used for further identification and for affinity purification of the adhesin part of the interaction, by streptavidin (Fig. 4A, B).

A multi-functional crosslinking agent with a biotin donating handle was attached to the Le^b glycoconjugate. The receptor activity of the Le^b glycoconjugate subsequently directed the targeted biotin tagging of the BabA adhesin protein (Fig. 4A, B). After crosslinking, the bacterial protein from strains A5, P466, and CCUG 17875 were separated on SDS-PAGE. Immunodetection with streptavidin demonstrated a biotin tagged protein, with the molecular weight of 74 kDa (Fig. 3C) (28), These results support the estimates of the molecular weight from the previous overlay analyses (Fig. 3B). Strain MO19 devoid of Le^b antigen binding properties (Fig. 3B) (Fig. 1A), was negative for binding also in this set of analyses (Fig. 3C).

The high specificity in the ReTagging technique provided a method for purification of the adhesin protein. Strains CCUG 17875 and A5, that both express the BabA adhesin (Fig. 1A) were processed by the ReTagging technique using crosslinker labelled Le^b receptor conjugate as the biotin donor. After crosslinking, bacteria were suspended in SDS sample buffer. Streptavidin coated magnetic beads were subsequently added to the solubilised proteins, and biotin tagged BabA was extracted (Fig. 4C). The N-terminal 20 amino acid sequences of the BabA adhesins from strains CCUG 17875 (Australia) and A5 (Sweden) were found to be identical, indicating a biologically conserved protein (Fig. 5). Recently, a series of outer membrane proteins from *H. pylori* were characterized. These proteins, HopA-E, are homologous in their N-terminal sequences to BabA (17), possible indicating a motif for a common secretion mechanism. The biotin tagged BabA adhesin was purified more than 3000-

fold from the cell extract, and the yield was calculated to 20%. However, based on data fr m the Scatchard plots, the level of available BabA adhesin would be about 5-times higher, i.e. approximately 1 mg adhesin/750 mg bacterial protein, which nevertheless could be the reason for the high signal to noise ratio (Fig. 3B). The purification of BabA via the ReTagging technique indicates the potential of this technique for the purification of lectins in complex receptor-ligand interactions, such as the selectin family of cell adhesion molecules.

To clone the gene encoding BabA, the N-terminal 20 aa sequence was utilised for the construction of degenerate primers (18). Two sets of clones were identified which both encode two different but very similar proteins. Both genes code for proteins having almost identical N-terminal domains and identical C-terminal domains, complicating the identification of the functional BabA gene. (Fig. 5). To identify the corresponding gene, the BabA adhesin was purified in large scale by ReTagging. This provided enough protein for an extended amino terminal sequence. 41 amino acids were identified and these residues unambiguously discriminated between the two genes by the differences in aa-positions 28, 35, 37, 38 and 41 (Fig. 5). The gene encoding BabA was named *babA* and correspond to a basic protein with a pI of 9.4 and a molecular weight of 78 kDa, i.e. of slightly higher molecular weight than that predicted from the SDS PAGE analyses (Fig. 3). The other gene, *babB*, corresponds to a protein of a calculated molecular weight of 75.5 kDa. In contrast to *babA*, the *babB* gene contains a predicted translational initiation codon (Fig. 5). This could indicate the existence of a third *bab* gene in the genome or mechanisms for recombination activities. Interestingly, the *bab*-genes were also detected in strains lacking Lewis b binding properties (data not shown). Gene cassette systems have been shown to promote antigenic variation in *Neisseria gonorrhoeae* (19). Another possibility would be the presence of similar genes coding for adhesins with differences in receptor specificity/host tissue tropism (20). Gene inactivation experiments targeting the *bab*-genes could aid in understanding this complex gene organisation.

Immunisation experiments with adhesins from *Bordetella pertussis* (21) indicate the potential for outer membrane proteins to act as vaccine candidates (discussed in ref. 22). In a mouse model for persistent *H. pylori* infection, oral immunisation with *H. pylori* antigens proved protective against *H. pylori* infection (10). However, results from animal models are difficult to evaluate for human specific pathogens, such as *H. pylori* and Polio virus. For Polio, an animal model has been achieved by expressing the virus receptor in transgenic mice (23). A similar strategy was taken for *H. pylori*. A transgenic mouse was constructed by the use of an a1,3/4-fucosyltransferase, driving the synthesis of the human specific Le^b antigen in

the gastrointestinal tract (24). The Lewis b mouse can be useful for the evaluation of the role of the BabA adhesin as a colonisation/virulence factor and in addition for the evaluation of BabA as a vaccine candidate against acid peptic disease and gastric adenocarcinoma.

In the present study the ReTagging technique was used for the purification of the adhesin part of the microbial receptor-ligand interaction. By the use of purified adhesin/lectin-protein, the ReTagging technique could, in addition, be used to further study the receptor part of the interaction. Identification of the biologically active receptor structure, carrying Le^b oligosaccharides, would aid in the understanding of the mechanisms supporting the chronic *H. pylori* infection.

Inhibition of *H. pylori* binding to ^{125}I-labeled Lewis b antigen by preparations is presented graphically, as a function of antibody concentration (mg/ml) in Fig. 6: 1 ml aliquots of *H. pylori* bacteria (A_{600}= OD 0.10) were pre-incubated with dilution series of antibody preparations, in 0.01-10 mg/ml for 2 hours in phosphate buffered saline (PBS), 0.5 % albumin, 0.05 % Tween-20. Then 500 ng of ^{125}I-labeled conjugate (i.e. an excess of receptor structure) was added and incubated for 30 minutes. After centrifugation, ^{125}I-activity in the bacterial pellet was measured by gamma scintillation counting. The Lewis b blood group antigen glycoconjugates used, i.e. semi-synthetic glycoproteins constructed by the conjugation of purified fucosylated oligosaccharides to serum albumin were from IsoSep AB, Tullinge, Sweden.

Western blot detection of the BabA adhesin by the different antibody preparations is presented in Fig. 7: Molecular weight rainbow marker (2 μL) from Amersham, Buckinghamshire, England, was dissolved in SDS sample buffer (lane 1). Approx. 100 ng of purified BabA adhesin (approx. 74 kDa with degradation product of approx. 55 kDa) was dissolved in SDS sample buffer (lane 2). SDS solubilized protein extracts of strain CCUG 17875 were prepared by dissolving the bacterial pellet corresponding to 0.15 ml of bacteria (A_{600}= OD 0.10) by SDS sample buffer (lane 3). The 3 protein samples were then boiled at 100°C for 5 minutes. The proteins were separated on SDS-PAGE, and transferred to a PVDF-membrane for Western blot immuno analysis. Five sets of PVDF-membranes were prepared. The PVDF membranes were blocked/incubated overnight with 4% human sera/plasma, in phosphate buffered saline, from a patient with no H. pylori infection, i.e. with no serum antibodies against H. pylori. The membrane was then washed in phosphate buffered saline (PBS), 0.5 % albumin, 0.05

% Tween-20, followed by the addition of the antibody preparations. The sets of membranes were incubated with the following 5 antibody preparations; 1) pooled human sera from H. pylori infected patients, diluted 1:500. 2) Chicken antibodies (positive) 1 mg/ml diluted 1:100x, 3) Bovine I preparation of antibodies, 1mg/ml diluted 1:100x. 4) Bovine II preparation of antibodies, 1mg/ml diluted 1:100x. 5) Bovine III preparation of antibodies, 1mg/ml diluted 1:100x (indicated in the figure). These antibodies were incubated with the membrane for 2 hours followed by extensive washings in phosphate buffered saline (PBS), 0.05 % Tween-20, followed by the addition of secondary anti-human, anti-chicken, and anti-bovine antibodies labeled with HRP-peroxidase (from DAKO, Denmark), all diluted 1:2000x. Membranes were incubated for 1 hour, followed by extensive washings in phosphate buffered saline (PBS), 0.05 % Tween-20. The membranes were developed with enhanced chemoluminescens (ECL) from Amersham. The results show, that the antigenic response against the adhesin is strongly enchanced in the bovine preparations. This finding is also supported by the inhibition data in Fig. 6.

Western blot analyses of *H. pylori* proteins by the different antibody preparations are shown in Fig. 8. 2 clinical isolates (1-2) from Dr. Lars Engstrand, Department of Clinical Microbiology and Cancerepidemiology, University Hospital, Uppsala, Sweden and strain CCUG 17875 (3), from Culture Collection, University of Göteborg, Department of Clinical Bacteriology, Göteborg, Sweden, and strain 52 (4) from Prof. Torkel Wadström, Dept. Medical Microbiology, Lunds University, were prepared for SDS-PAGE electrophoresis. Bacterial pellets corresponding to 0.15 ml of bacteria (A_{600}= OD 0.10) were dissolved in SDS sample buffer and heated to 100°C for 5 minutes. The proteins were separated on SDS-PAGE, and transferred to PVDF-membranes for Western blot immuno analysis. The western blot analyses were as described above, i.e. the sets of membranes were incubated with the following 4 antibody preparations; 1) pooled human sera from *H. pylori* infected patients, diluted 1:500. 2) Chicken antibodies (positive) 1mg/ml diluted 1:100x, 3) Bovine I preparation of antibodies, 1mg/ml diluted 1:100x. 4) Bovine III preparation of antibodies, 1mg/ml diluted 1:100x (indicated in the figure). These antibodies were incubated with the membrane for 2 hours followed by extensive washings in phosphate buffered saline (PBS), 0.05 % Tween-20, followed by the addition of secondary anti-human, anti-chicken, and anti-bovine antibodies labeled with HRP-peroxidase (from DAKO, Denmark), all diluted 1:2000x. Membranes were incubated for 1 hour, followed by

extensive washings in phosphate buffered saline (PBS), 0.05 % Tween-20. The membranes were developed with enhanced chemoluminescens (ECL) from Amersham. The results show, that the chicken antibodies and the bovine preparations reacts nearly identically against all four strains, indicating conserved properties in strains of different geographical origin.

Although the invention has been described with regard to its preferred embodiments, which constitute the best mode presently known to the inventors, it should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in the claims appended hereto.

REFERENCES AND NOTES

1. J. R. Warren, *Lancet*, i, 1273, 1983, B. Marshall, *Lancet*, i, 1273, 1983.

2. A. Dubois, *Emerging Infectious Diseases* **1**, 79 (1995).

3. M. J. Blaser, *Sci. Amer.* **2**, 92 (1996).

4. 3. M. J. Blaser, *Trends Microbiol.* **7**, 255 (1993), D. E. Kirschner and M. J. Blaser, *J. Theor. Biol.* **176**, 281 (1995).

5. P. Falk, T. Borén, and S. Normark, *Meth. Enzymol.* **236**, 353 (1994).

6. D. G. Evans, D. J. Evans Jr., J. J. Moulds and D. Y. Graham, *Infect. Immun.* **56**, 2896 (1988), S. Hirmo, M. Utt, M. Ringner and T. Wadström, *FEMS Immunol. and Med. Microbiol.* **10**, 301 (1995), T. Saitoh, et al *FEBS Lett.* **282**, 385 (1991).

7. T. Borén, P. Falk, K. A. Roth, G. Larson, and S. Normark, *Science*. **262**, 1892 (1993), P. Falk, et al *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2035 (1993).

8. *H. pylori* strain CCUG 17875 was obtained from CCUG, Göteborg, Sweden. Strain A5, a gastric ulcer isolate, came from Astra Arcus, Södertälje, Sweden. Strains P466 and MO19 were described previously (7). Strain 26695 came from Dr. K A. Eaton, The Ohio State University, and its genome was recently sequenced by The Institute for Genomic Research (TIGR), Rockville, Maryland (J.-F. Tomb, et al, abstract 3B: 059, IX International Workshop on Gastroduodenal Pathology and *Helicobacter pylori*, Copenhagen, Denmark, 1996). The panel of 45 *H. pylori* clinical isolates came from the University Hospital in Uppsala, Sweden. Bacteria were grown at 37(C in 10 % CO_2 and 5% O_2 for 48 h.

9. All blood group antigen glycoconjugates used, i.e. semi-synthetic glycoproteins constructed by the conjugation of purified fucosylated oligosaccharides to serum albumin (7, 25), were from IsoSep AB, Tullinge, Sweden. The RIA was performed according to ref. 26 with some modifications; The H-1, Le^b, Le^a, H-2, Le^x, and Le^y glycoconjugates were ^{125}I-labeled by the Chloramine T method. 1 ml of bacteria (A_{600}= OD 0.10) was incubated with 300 ng of ^{125}I-labeled conjugate (i.e. an excess of receptors) for 30 min. in phosphate buffered saline (PBS), 0.5 % albumin, 0.05 % Tween-20 (BB-buffer). After centrifugation, ^{125}I-activity in the bacterial pellet was measured by gamma scintillation counting.

10. A. Covacci, et al, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 5791 (1993), M. Marchetti, et al, *Science* **267**, 1655 (1995).

11 S. Censini et al, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 14648; (1996).

12. Z. Xiang, et al, *Infect. Immun.* **63**, 94 (1995).

13. A. G. Scatchard, *Ann. N. Y. Acad. Sci.* **51**, 600 (1949).

14. O. Mol, and B. Oudega, *FEMS. Microbiol. Reviews*, **19**, 25 (1996).

15. Confocal microscopy was performed on a Nikon/Multiprobe 2001 instrument (Molecular Dynamics, Sunnyvale, CA). Electron microscopy was performed on a JEOL 100 CX instrument.

16. J. Brunner, *Trends in Cell Biol.* **6**, 154 (1996), J. D. Bleil and P. M. Wassarman, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5563, (1990).

17. M. M. Exner, P. Doig, T. J. Trust, and R. E. W. Hancock, *Infect. Immun.* **63**, 1567 (1995), P. Doig, M. M. Exner, R. E. W. Hancock and T. J. Trust, *J. Bacteriol.* **177**, 5447 (1995).

18. The BabA N-terminal sequence analysis was used to make degenerate oligonucleotides, which were used in PCR to obtain an amplified fragment from the chromosome of the *babA* gene. A 59 bp fragment was identified and used as probe for the screening of a low-copy plasmide (pACYC184) library of *Sau3A* partially digested chromosomal DNA from strain CCUG 17875.

19. P. Hagblom, E. Segal, E. Billyard, and M. So, *Nature*, **315**, 156 (1985), R. Haas and T. F. Meyer, *Cell*, **44**, 107 (1986).

20. A.-B. Jonsson, D. Ilver, P. Falk, J. Pepose, and S. Normark, *Mol. Microbiol*, **13**, 403 (1994), N. Strömberg, P. G. Nyholm, I. Pascher, and S. Normark, *Proc. Natl. Acad. Sci USA* **88**, 9340 (1991).

21. A. Kimura, K. T. Mountzouros, D. A. Relman, S. Falkow, J. L. Cowell, *Infect. Immun.* **58**, 7 (1990).

22. T. Borén, and P. Falk, *Sci. Amer., Sci. & Med.* 4 (1994), L. S. Tompkins and S. Falkow, Science **267**, 1621 (1995).

23. R. B. Ren, et al, *Cell* **63**, 353 (1990).

24. P. G. Falk, L. Bry, J. Holgersson, and J. I. Gordon, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 1515 (1995).

25. P. D. Rye, *Nature Biotechnology.* **2**, 155 (1996).

26 P. Falk, T. Borén, D. Haslam, M. G. Caparon, *Meth. Cell Biol.* **45**, 161 (1994)

27. Cell extracts were prepared in SDS sample buffer without mercapto ethanol and heated at 37°C or 97°C for 10 min. before separation on SDS-PAGE. Proteins were blotted onto a PVDF membrane. The membrane was incubated with 1μg /ml biotinylated Le^b glycoconjugate or biotinylated albumin (negative control) overnight, labelled as described in ref. 7. After washing in PBS/0.05% Tween-20, the biotinylated structures bound by the BabA band were probed by HRP-streptavidin and detected using ECL reagents (Amersham, Buckinghamshire, England).

28. The bacterial suspension was incubated with Le^b glycoconjugate, to which the Sulfo-SBED crosslinker (Pierce, Rockville, IL.) had been conjugated by the N- hydroxysuccinimide ester (NHS), according to the manufacturers specifications. The aryl azide crosslinker group was activated by UV irradiation (360 nm). Bacteria were washed with PBS pH 7.6, 0.05 % Tween-20 and protease inhibitors (EDTA and benzamidine) under reducing conditions with 50 mM dithiothreitol (DTT). Bacterial proteins were separated on SDS-PAGE, and the biotin tagged BabA protein was detected by immunodetection (PVDF membrane/ HRP-streptavidin and ECL) (Fig. 3C).

29. Strains CCUG 17875 and A5 were first processed by crosslinking and DTT treatment, as above (28), followed by solubilisation in SDS sample buffer. The biotin tagged BabA protein was then extracted with streptavidin coated magnetic beads (Advanced Magnetics Inc., Cambridge, MA). The beads were boiled in SDS sample buffer, and bound proteins were eluted and alkylated. The protein preparation was further fractionated by preparative SDS-PAGE (Prep-Cell 491, BioRad, Hercules, CA). Fractions with the biotin tagged protein, i.e. the BabA fractions, were identified by immunodetection using streptavidin/ECL. The pooled BabA preparation was then separated on SDS-PAGE and transferred to PVDF membrane. The BabA band was excised and the BabA protein was N-terminally sequenced using a Procise™ 494 instrument (Applied Biosystems, Foster City, CA).

Claims

1. A novel bacterial blood group antigen binding (BAB) adhesin protein in *Helicobacter pylori* species and fractions of said protein, **characterized** in that said protein or fractions thereof bind specifically to fucosylated blood group antigens.

2. A substantially pure adhesin protein and fractions thereof according to claim 1, **characterized** in that said protein contains less than 20 % bacterial protein impurities.

3. Adhesin protein and fractions thereof according to claim 2, **characterized** in that the following amino acid sequence is included:

EDDGFYTSVGYQIGEAAQMV

and homologues thereof.

4. Adhesin protein and fractions thereof according to claim 3, **characterized** in that the following amino acid sequence:

EDDGFYTSVGYQIGEAAQMV

or homologues thereof is in an amino terminal position.

5. Adhesin protein and fractions thereof according to any one of claim 1 to 4, **characterized** in that the unfractionated protein has a molecular weight in the interval about 70 to 77 kDa, preferably in the interval of 73 to 75 kDa as determined by SDS-PAGE.

6. Adhesin protein and fractions thereof according to any one of claim 1 to 5, **characterized** in that the unfractionated protein has a molecular weight of about 73.5 kDa as determined by SDS-PAGE.

7. Adhesin protein and fractions thereof according to claim 6, **characterized** in that said protein is specific for at least one of the *Helicobacter pylori* strains included in the following group: CCUG 17875, NCTC 11637, A5, P466, G109, G56, Ba 185, Ba 99, 931 and 932.

8. Adhesin protein and fractions thereof according to claim 6, **characterized** in that said protein is specific for all the *Helicobacter pylori* strains included in the following group: CCUG 17875 and A5.

9. Pharmaceutical composition **characterized** in that it comprises a pharmaceutically effective amount of an adhesin protein or fractions thereof according to any one of claims 1 to 8, optionally together with pharmaceutically acceptable excipients.

10. DNA for expression of an adhesin protein or fractions thereof according to any one of the preceeding claims.

11. DNA for expression of an adhesin protein or fractions thereof according to claim 10, **characterized** in that it includes the following sequence:

5´- GAAGACGACGGCTTTTACACAAGCGTAGGCTATCAAATCGGTGAAGCCGCT-CAAATGGTA - 3´

or homologues thereof.

12. DNA for expression of an adhesin protein or fractions thereof according to claim 10, **characterized** in that it includes the following sequence:

see Appendix 1

or homologues thereof.

13. DNA for expression of an adhesin protein or fractions thereof according to claim 10, **characterized** in that it includes the following sequence:

see Appendix 2

or homologues thereof.

14. Method for isolation and purification of an adhesin protein according to any one of claims 1 to 8, **characterized** in that the method includes the following steps:

- labelling Lewis^b neoglycoconjugate with a biotin-donating crosslinker agent,
- incubation of the bacteria with crosslinker labelled Lewis^b conjugate,
- solubilising the BAB-adhesin,
- extraction of the biotin tagged BAB-adhesin using streptavidin coated magnetic beads, and
- purification of the resulting adhesin-fraction using SDS-PAGE.

15. Monospecific antisera produced using a pure adhesin protein or fractions thereof according to any one of claims 1 to 8.

16. Use of monospecific antisera produced according to claim 15, for quantitative or qualitative determinations of adhesin protein or fractions thereof in cells, tissues or body fluids.

17. A test kit comprising monospecific antisera produced according to claim 15, in combination with conventional markers and reagents.

18. Therapeutic treatment of infections of the gastrointestinal mucous membrane, **characteriz d** in that an adhesin protein or fractions thereof according to any one of

claims 1 to 8 is/are administered orally to a patient to raise antibodies against an infectious agent.

19. Immunisation therapy for prevention of infections of the gastrointestinal mucous membrane, **characterized** in that an adhesin protein or fractions thereof according to any one of claims 1 to 8 is/are used to raise antibodies against an infectious agent.

20. Immunisation therapy for prevention of infections of the gastrointestinal mucous membrane, **characterized** in that DNA according to any one of claims 10 to 11 is used to raise antibodies against an infectious agent.

21. Therapeutic treatment according to claim 18, **characterized** in that said infectious agent is *Helicobacter pylori*.

22. Immunisation therapy according to claim 19, **characterized** in that said infectious agent is *Helicobacter pylori*.

23. Immunisation therapy according to claim 20, **characterized** in that said infectious agent is *Helicobacter pylori*.

24. Vaccine composition for the treatment of *Helicobacter pylori* infection, **characterized** in that it comprises a pharmaceutically acceptable excipient and an effective amount of an adhesin protein or fractions thereof according to any one of claims 1 to 8.

25. Use of an adhesin protein or fractions thereof according to any one of claims 1 to 8, for the manufacture of a vaccine.

26. Use of DNA according to any one of claims 10 to 13, for the manufacture of a vaccine.

27. Use of monospecific antisera according to claim 15, for the manufacture of a test kit.

28. Immunoglobulin composition, **characterized** in that said composition exhibits specific activity to a Lewis^b binding adhesin protein or fractions thereof, expressed by *Helicobacter pylori*.

29. Immunoglobulin composition according to claim 28, **characterized** in that said adhesin in its unfractionated form has a molecular weight in the interval of about 70 to 77 kDa, preferably in the interval of 73 to 75 kDa and most preferably about 73500 kDa, as determined by SDS-PAGE.

30. Immunoglobulin composition according to any one of claim 1 - 2, **characterized** in that said adhesin or fractions thereof comprises the following amino acid sequence:

EDDGFYTSVGYQIGEAAQMV

or homologues thereof.

31. Antibody, **characterized** in that said antibody exhibits specific activity to a Lewis^b binding adhesin protein or fractions thereof, expressed by *Helicobacter pylori*.

32. Antibody according to claim 31, **characterized** in that said adhesin in its unfractionated form has a molecular weight in the interval of about 70 to 77 kDa, preferably in the interval of 73 to 75 kDa and most preferably about 73500 kDa, as determined by SDS-PAGE.

33. Antibody according to any one of claim 31 - 32, **characterized** in that said adhesin or fractions thereof comprises the following amino acid sequence:

EDDGFYTSVGYQIGEAAQMV

or homologues thereof.

34. Antibody according to any one of claims 31 - 33, **characterized** in that said antibody is a monoclonal antibody.

35. Method of manufacturing an immunoglobulin composition according to any one of claim 28 - 30, **characterized** in that said method comprises the following steps:

- immunising an animal with a Lewis^b binding adhesin protein or fractions thereof, expressed by *Helicobacter pylori*,
- isolation of the immunoglobulin fraction from an excretion of said host animal,
- purification of the immunoglobulin preparation.

36. Method according to claim 35, **characterized** in that said animal is a cow and the immunoglobulin fraction is isolated from the milk, preferably the colostrum thereof.

37. Method according to claim 35, **characterized** in that said animal is a chicken and the immunoglobulin fraction is isolated from the egg yolk thereof.

38. Method of manufacturing an antibody according to any one of claim 31 - 34, **characterized** in that said method comprises the following steps:

- immunising an animal with a Lewis^b binding adhesin protein or fractions thereof, expressed by *Helicobacter pylori*,
- fusing immunised, immunoglobulin producing cells with a neoplastic cell line,
- selection and growing of cells expressing said antibody,
- purification of the antibodies.

39. Method of manufacturing an antibody according to any one of claim 31 - 34, **characterized** in that said antibody is expressed by a culture of viable micro-organisms in an expression system, where said micro-organism or organisms is/are generally recognised as safe (GRAS) and genetically modified to express said antibody.

40. Method according to claim 39, **characterized** in that said micro-organism is chosen from the group comprising bacteria of the species *Lactobacillus*, *Staphylococcus* and *Enteriobacteriacea*.

41. Use of an immunoglobulin composition according to any one of claim 28 - 30 for the manufacture of a pharmaceutical preparation for the treating and/or preventing *Helicobacter pylori* infection in humans.

42. Use of an immunoglobulin composition according to any one of claim 28 - 30 for the manufacture of a pharmaceutical product for the treating and/or preventing gastric ulcers.

43. Use of an immunoglobulin composition according to any one of claim 28 - 30 for the manufacture of a pharmaceutical product for the treating and/or preventing acid peptic disease.

44. Use of an antibody according to any one of claim 31 - 34 for the manufacture of a pharmaceutical product for the treating and/or preventing *Helicobacter pylori* infection in humans.

45. Use of an antibody according to any one of claim 31 - 34 for the manufacture of a pharmaceutical product for the treating and/or preventing gastric ulcers.

46. Use of an antibody according to any one of claim 31 - 34 for the manufacture of a pharmaceutical product for the treating and/or preventing acid peptic disease.

47. A method for treating and/or preventing *Helicobacter pylori* infections in humans, said method comprising orally administering an effective amount of an immunoglobulin composition according to any one of claim 28 - 30.

48. A method for treating and/or preventing *Helicobacter pylori* infections in humans, said method comprising orally administering an effective amount of an antibody according to any one of claim 31 - 34.

49. A method for treating and/or preventing *Helicobacter pylori* infections in humans, said method comprising orally administering an effective amount of a culture of

viable micro-organisms in an expression system, where said micro-organism or organisms is/are generally recognised as safe (GRAS) and genetically modified to express either an antibody according to any one of claims 33 to 34 or the adhesin protein according to any one of claims 1 to 8.

50. Use of a culture of viable micro-organisms in an expression system, where said micro-organism or organisms is/are generally recognised as safe (GRAS) and genetically modified to express an antibody according to any one of claims 33 – 34 for the manufacture of an oral composition for the treatment or prevention of *Helicobacter* infection in humans.

------

babA APPENDIX 1

```
   1  TTTCAGTCAA GCCCAAAGCT ATGCGCAAAA CGCTTATGCT AAAGAGAATT
  51  TACAAGCACA GCCGTCCAAG TATCAAAACA GCGTGCCTGA AATCAATATT
 101  GATGAAGAAG AAATCCCCTT TAAGGGTTAA AATTAAGGAG ACATTATGGA
 151  AAGAAAACGC TATTCAAAAC GCTATTGCAA ATACACTGAA GCTAAAATCA
 201  GCTTTATTGA CTATAAAGAT TTGGACATGC TCAAGCACAC GCTATCAGAG
 251  CGCTATAAAA TCATGCCAAG GAGGTTGACA GGCAATAGCA AAAAGTGGCA
 301  AGAGAGGGTG GAAGTTAGCG ATCAAAAGAG CCCGCCACAT GGCTTTAATC
 351  CCCTACATTG TGGATAGGAA AAAAGTCGTG GATAGCCCTT TTAAACAGCA
 401  CTGAATTTTT GATTAGGGCT AATAGGGGGC ATGCCTTTTA ATCTTGTTTA
 451  ATCTTGGCTC TATTTTTGTT AAACATCGGT TATAAAAGCG TTAAAAGCAC
 501  TTTTAAAATC CAATTAAAAG CGTTCAAAAG TAACGCAAAA AATCAAAAAA
 551  ATGACAAAAT TTTTAAGAAA ATGACAAAAA AAAAAAAAAC GCTTTATGCT
 601  ATAATATTCC AAATACATTC TAATGCAAAT GCATTCTAAT GCAAATGTAT
                                             ->ORF-start
 651  AATGAATGTA TGAAATCCCT AATATTCAAT CCAATTTAAT CCAAAAAGGA
 701  GAAAAAACAC ATCCTTTCAT TAACTTTAGG CTCGCTTTTA GTTTCCACTT
 751  TGAGCGCTGA AGACGACGGC TTTTACACAA GCGTAGGCTA TCAAATCGGT
 801  GAAGCCGCTC AAATGGTAAC AAACACCAAA GGCATCCAAG ATCTTTCAGA
 851  CAACTATGAA AACTTGAGCA AACTTTTGAC CCGATACAGC ACCCTAAACA
 901  CCCTTATCAA ATTGTCCGCT GATCCGAGCG CGATTAACGC GGCACGTGAA
 951  AATCTGGGCG CGAGCGCGAA GAACTTGATC GGCGATACCA AAAATTCCCC
1001  CGCCTATCAA GCCGTGCTTT TGGCGATCAA TGCGGCGGTA GGGTTTTGGA
1051  ATGTCTTAGG CTATGCTACG CAATGCGGGG GTAACGCTAA TGGTCAAGAA
1101  AGCACCTCTT CAACCACCAT CTTCAACAAC GAGCCAGGGT ATCGATCCAC
1151  TTCCATCACT TGCAGTTTGA ACAGGTATAA GCCTGGATAC TACGGCCCTA
1201  TGAGCATTGA AAATTTCAAA AAGCTTAACG AAGCCTATCA AATCCTCCAA
1251  ACGGCTTTAA ATAAAGGCTT ACCCGCGCTC AAAGAAAACA ACGGAACGGT
```

APPENDIX 1

1301 CAGTGTAACC TACACCTACA CATGCTCAGG GGAAGGGAAT GATAACTGCT
1351 CGAAAAAAGC CACAGGTGTA AGTGACCAAA ATGGCGGAAC CAAAACTAAA
1401 ACCCAAACCA TAGACGGCAA AACCGTAACC ACCACGATCA GTTCAAAAGT
1451 CGTTGATAGT CAGGCAAAAG GTAATACAAC AAGGGTGTCC TACACCGAAA
1501 TCACTAACAA ATTAGACGGT GTGCCTGATA GCGCTCAAGC GCTCTTGGCG
1551 CAAGCGAGCA CGCTCATCAA CACCATCAAC ACGGCATGCC CGTATTTTAG
1601 TGTAACTAAT AAAAGTGGTG GTCCACAGAT GGAACCGACT AGAGGGAAGT
1651 TGTGCGGTTT TACAGAAGAA ATCAGCGCGA TCCAAAAGAT GATCACAGAC
1701 GCGCAAGAGC TGGTCAATCA AACGAGCGTC ATTAACGAGC ATGAACAATC
1751 AACCCCGGTA GGCGGTAATA ATGGCAAGCC TTTCAACCCT TTCACGGACG
1801 CAAGCTTCGC TCAAGGCATG CTCGCTAACG CTAGTGCGCA AGCCAAAATG
1851 CTCAATCTAG CCCATCAAGT GGGGCAAACC ATTAACCCTG ACAATCTTAC
1901 CGGGACTTTT AAAAATTTTG TTACAGGCTT TTTAGCCACA TGCAACAACA
1951 AATCAACAGC TGGCACTAGT GGCACACAAG GTTCACCTCC TGGCACAGTA
2001 ACCACTCAAA CTTTCGCTTC CGGTTGCGCC TATGTGGAGC AAACCATAAC
2051 GAATCTAAAC AACAGCATCG CTCATTTTGG CACTCAAGAG CAGCAGATAC
2101 AGCAAGCTGA AAACATCGCT GACACTCTAG TGAATTTCAA ATCTAGATAC
2151 AGCGAATTAG GGAATACTTA TAACAGCATC ACTACTGCGC TCTCCAAAGT
2201 CCCTAACGCG CAAAGCTTGC AAAACGTGGT GGGAAAAAAG AATAACCCCT
2251 ATAGCCCGCA AGGCATAGAA ACCAATTACT ACTTGAATCA AAACTCTTAC
2301 AACCAAATCC AAACCATCAA CCAAGAATTA GGGCGTAACC CCTTTAGGAA
2351 AGTGGGCATC GTCAGTTCTC AAACCAACAA TGGTGCCATG AATGGGATCG
2401 GTATCCAGGT GGGCTACAAG CAATTCTTTG GGCAAAAAAG GAAATGGGGT
2451 GCAAGATACT ACGGCTTTTT TGATTACAAC CATGCGTTCA TTAAATCCAG
2501 CTTCTTCAAC TCGGCTTCTG ACGTGTGGAC TTATGGTTTT GGAGCGGACG
2551 CTCTTTATAA CTTCATCAAC GATAAAGCCA CCAATTTCTT AGGCAAAAAC
2601 AACAAGCTTT CTGTGGGGCT TTTTGGCGGG ATTGCGTTAG CGGGCACTTC

APPENDIX 1

2651 ATGGCTTAAT TCTGAATACG TGAATTTAGC CACCATGAAT AACGTCTATA

2701 ACGCTAAAAT GAACGTGGCG AACTTCCAAT TCTTATTCAA CATGGGAGTG

2751 AGGATGAATT TAGCCAGATC CAAGAAAAAA GGCAGCGATC ATGCGGCTCA

2801 GCATGGCATT GAGTTAGGGC TTAAAATCCC CACCATTAAC ACGAACTACT

2851 ATTCCTTTAT GGGGGCTGAA CTCAAATACC GCAGGCTCTA TAGCGTGTAT

2901 TTGAATTATG TGTTCGCTTA CTAAAAACTA AAAATCCTTT GTGGAACTCC
ORF-stopp

2951 CTTTTTAAGG GGTTTCTTTT AAAGCCTTTA TTTTTTTTTG GAGGGGTTTA

3001 ATTTTTTTGA AACCTTTGTT TTTGAATTCT CTTTTTAATG GGTTTCTTTT

3051 TTGAACTCTT TGTTTTGAAC TCCTTTTTTT GAACTCCCTT TTTTAAACCC

3101 TTTCTTTTTT AAAATTCTCT TTTTTGGGGG GTTTGATGAA AAATCCTTTT

3151 TTAGCGTTTT GGTATTGGTT AGTGGAAAAC TTGATACTAA TTTAAGCGAT

3201 AGTTTTTAAA AAGTGCTTCT TTAATATAGG GGGTTTAAGT TGGTGATTAA

3251 AAGGGGGGAA TGGTTTCAAA GCGCTTCCTA TCCCTTTAAG AAAATAAAAT

3301 AAAACTTTAA TAAAATGAGT TTTACAACAA AATGAGATCC

APPENDIX 2

babB

1 CATTTGATCG CATTGGATTT CAAAGAAGGG CGTTTTGTGA AAGGCTTTGG

51 TCAAGCTTAT GATATTTTAG GCGACAAAAT CGCTTATGTT GGGGGTAAAG

101 GCAACCCACA CAATTTCGCT CACAAGAAAT AAACTTTCTC ACCCATAAGG

151 GGCAAACGCC CCCAAAAGAG TGCTTTTTAA AGAGGTTAAG GCAAAATCAA

201 GCTCTTTAGT ATTTAATCTT AAAAAATACT AAAAGCCTTT TTATGGGCTA

251 ACACCACACA AAAAGCGTCA AAATCAAAAA AATGACAAAA TTTTCCCCAA

301 ATGACAAAAA AAAAAAAAAA CGATTTTATG CTATATTAAC GAAATCTTGT

351 GATAAGATCT TATTCTTTTA AAAGATTTAC CTAACCATTT TAATTTCAAG

ORF-start

401 GAGAAAACAT GAAAAAAAAC CCTTTTACTC TCTCTCTCTC TCTCTCGTTT

451 TTGCTCCACG CTGAAGACGA CGGCTTTTAC ACAAGCGTAG GCTATCAAAT

501 CGGTGAAGCC GCTCAAATGG TAACCAACAC CAAAGGCATC CAACAGCTTT

551 CAGACAATTA TGAAAAGCTG AACAATCTTT TGAATAATTA CAGCACCCTA

601 AACACCCTTA TCAAATTATC CGCTGATCCG AGTGCGATTA ACGACGCAAG

651 GGATAATCTA GGCTCAAGTG CTAAGAATTT GCTTGATGTT AAAACCAACT

701 CCCCGGCCTA TCAAGCCGTG CTTTTAGCGT TGAATGCGGC GGTGGGGTTG

751 TGGCAAGTTA CAAGCTACGC TTTTACTGCT TGTGGTCCTG GCAGTAACGA

801 GAGCGCAAAT GGAGGTATCC AAACTTTTAA TAATGTGCCA GGACAAAAGA

851 CGACAACCAT CACTTGCAAT TCGTATTATC AACCAGGACA TGGTGGGCCT

901 ATATCCACTG CAAACTATGC AAAAATCAAT CAAGCCTATC AAATCATTCA

951 AAAGGCTTTG ACAGCCAATG AAGCTAATGG AGATGGGGTC CCCGTTTTAA

1001 GCGACACCAC TACAAAACTT GATTTCACTA TTCAAGGAGA CAAAAGAACG

1051 GGTGGCCGAC CAAATACACC TAAAAAGTTC CCATGGAGTG ATGGGAAATA

1101 TATTCACACC CAATGGATTG ACACAACACC ACAATCAACA GAAACAAAGA

1151 TCAACACAGA AAATAACGCT CAAGAGCTTT TAAAACAAGC GAGCATCATT

1201 ATCACTACCC TAAATGAGGC ATGCCCAAAC TTCCAAAATG GTGGTAGCGG

1251 TTATTGGCAA GGGATAAGCG GCAATGGGAC AATGTGTGGG ATGTTTAAGA

APPENDIX 2

1301 ATGAAATCAG CGCTATCCAA GGCATGATCG CTAACGCGCA AGAAGCTGTC

1351 GCGCAAAGTA AAATCGTTAG TGAAAATGCG CAAAATCAAA ACAACTTGGA

1401 TACTGGAAAA CCATTCAACC CTTTCACGGA CGCTAGCTTC GCTCAAAGCA

1451 TGCTCAAAAA CGCTCAAGCC CAAGCAGAGA TTTTAAACCA AGCCGAACAA

1501 GTGGTGAAAA ACTTTGAAAA AATCCCTAAA AATTTTGTAT CAGACTCTTT

1551 AGGGGTGTGT TATGAAGAGC AAGGGGGTGA GCGTAGGGGC ACCAATCCAG

1601 GTCAGGTTAC TTCTAACACT TTCGCTTCCG GTTGCGCCTA TGTGGAGCAA

1651 ACCATAACGA ATCTAAACAA CAGCATCGCT CATTTTGGCA CTCAAGAGCA

1701 GCAGATACAG CAAGCTGAAA ACATCGCTGA CACTCTAGTG AATTTCAAAT

1751 CTAGATACAG CGAATTAGGG AATACTTATA ACAGCATCAC TACTGCGCTC

1801 TCCAAAGTCC CTAACGCGCA AAGCTTGCAA AACGTGGTGG GAAAAAAGAA

1851 TAACCCCTAT AGCCCGCAAG GCATAGAAAC CAATTACTAC TTGAATCAAA

1901 ACTCTTACAA CCAAATCCAA ACCATCAACC AAGAATTAGG GCGTAACCCC

1951 TTTAGGAAAG TGGGCATCGT CAGTTCTCAA ACCAACAATG GTGCCATGAA

2001 TGGGATCGGT ATCCAGGTGG GCTACAAGCA ATTCTTTGGG CAAAAAAGGA

2051 AATGGGGTGC AAGATACTAC GGCTTTTTTG ATTACAACCA TGCGTTCATT

2101 AAATCCAGCT TCTTCAACTC GGCTTCTGAC GTGTGGACTT ATGGTTTTGG

2151 AGCGGACGCT CTTTATAACT TCATCAACGA TAAAGCCACC AATTTCTTAG

2201 GCAAAAACAA CAAGCTTTCT GTGGGGCTTT TTGGCGGGAT TGCGTTAGCG

2251 GGCACTTCAT GGCTTAATTC TGAATACGTG AATTTAGCCA CCATGAATAA

2301 CGTCTATAAC GCTAAAATGA ACGTGGCGAA CTTCCAATTC TTATTCAACA

2351 TGGGAGTGAG GATGAATTTA GCCAGATCCA AGAAAAAAGG CAGCGATCAT

2401 GCGGCTCAGC ATGGCATTGA GTTAGGGCTT AAAATCCCCA CCATTAACAC

2451 GAACTACTAT TCCTTTATGG GGGCTGAACT CAAATACCGC AGGCTCTATA

2501 GCGTGTATTT GAATTATGTG TTCGCTTACT AGAAACTAAA AATCCTTTGT

ORF-stopp

2551 GGAACTCCCT TTTTAAGGGG TTTCTTTTAA AGCCTTTATT TTTTTTTGGA

SUBSTITUTE SHEET

APPENDIX 2

2601 GGGGTTTAAT TTTTTTGAAA CCTTTGTTTT TGAATTCTCT TTTTAATGGG

2651 TTTCTTTTTT GAACTCTTTG TTTTGAACTC CTTTTTTTGA ACTCCCTTTT

2701 TTAAACCCTT TCTTTTTTAA AATTCTCTTT TTTGGGGGGT TTGATGAAAA

2751 ATCCTTTTTT AGCGTTTTGG TATTGGTTAG T

Alignment of translated sequences of the *babA* and *babB* genes

APPENDIX 3

```
   -23                                   1
    ▼                                    ▼
BabA SKKEKKHILSLTLGSLLVSTLSAEDDGFYTSVGYQIGEAAQMVTNTKGIQ
      .|... .|||.|: |    | |||||||||||||||||||||||||||
BabB  MKKNPFTLSLSLSFL....LHAEDDGFYTSVGYQIGEAAQMVTNTKGIQ
      ▲
    -18

                  41  43
                   ▼   ▼
     DLSDNYENLSKLLTRYSTLNTLIKLSADPSAINAARENLGASAKNLIGDT
     :||||||.|..||..||||||||||||||||||.||:|||.||||||:: .
     QLSDNYEKLNNLLNNYSTLNTLIKLSADPSAINDARDNLGSSAKNLLDVK

     79
     ▼
     KNSPAYQAVLLAINAAVGFWNVLGYATQCGGNANGQESTSSTTIFNNEPG
     .||||||||||||:|||||:|.| :||   ..| :..:...::. .||| ||
     TNSPAYQAVLLALNAAVGLWQVTSYAFTACGPGSNESANGGIQTFNNVPG

     YRSTSITCSLNRYKPGYYGPMSIENFKKLNEAYQILQTALNKGLPALKEN
      :.|.|||. .  |.||. ||:|..|: |:|:||||:|    |:|.| ..|
     QKTTTITCN.SYYQPGHGGPISTANYAKINQAYQIIQ....KALTANEAN

     NGTVSVTYTYTCSGEGNDNCSKKATGVSDQNGGTKTKTQTIDGKTVTTTI
     .:.|.|  . |.. : . ...|:..|     ...|..| . ||| : |
     GDGVPVLSDTTTKLDFTIQGDKRTGG....RPNTPKKPPWSDGKYIHT..

     SSKVVDSQAKGNTTRVSYTEITNKLDGVPDSAQALLAQASTLINTINTAC
       . :|. :.:..|::            ...:.||.|| |||.:|.|:|.||
     ..QWIDTTPQSTETKI..........NTENNAQELLKQASIIITTLNEAC

     PYFSVTNKSGGPQMEPTRGKLCG.FTEEISAIQKMITDAQELVNQTSVIN
     | |   .. ||  |   ...|.:|| |.:|||||| ||.:||| |.|..::.
     PNFQ.NGGSGYWQGISGNGTMCGMFKNEISAIQGMIANAQEAVAQSKIVS

               339
                ▼
     EHEQSTPVGGNNGKPFNPFTDASFAQGMLANASAQAKMLNLAHQVGQTIN
     |:.|. . . :.||||||||||||||||:|| || |||.:|| |.||..
     ENAQN.QNNLDTGKPFNPFTDASFAQSMLKNAQAQAEILNQAEQVVK...
```

Continue ...

Continue

Alignment of translated sequences of the *babA* and *babB* genes

APPENDIX 3

```
                                        418
                                         ▼
PDNLTGTFKNFVTGFLATCNNKSTAGTSGTQGSPPGTVTTQTFASGCAYV
  |:.  .||||.: |:.| :.  .:: .||.  ||  ||..|||||||||
..NFEKIPKNFVSDSLGVCYEEQGGERRGTN...PGQVTSNTFASGCAYV

EQTITNLNNSIAHFGTQEQQIQQAENIADTLVNFKSRYSELGNTYNSITT
||||||||||||||||||||||||||||||||||||||||||||||||||
EQTITNLNNSIAHFGTQEQQIQQAENIADTLVNFKSRYSELGNTYNSITT

ALSKVPNAQSLQNVVGKKNNPYSPQGIETNYYLNQNSYNQIQTINQELGR
||||||||||||||||||||||||||||||||||||||||||||||||||
ALSKVPNAQSLQNVVGKKNNPYSPQGIETNYYLNQNSYNQIQTINQELGR

NPFRKVGIVSSQTNNGAMNGIGIQVGYKQFFGQKRKWGARYYGFFDYNHA
||||||||||||||||||||||||||||||||||||||||||||||||||
NPFRKVGIVSSQTNNGAMNGIGIQVGYKQFFGQKRKWGARYYGFFDYNHA

FIKSSFFNSASDVWTYGFGADALYNFINDKATNFLGKNNKLSVGLFGGIA
||||||||||||||||||||||||||||||||||||||||||||||||||
FIKSSFFNSASDVWTYGFGADALYNFINDKATNFLGKNNKLSVGLFGGIA

LAGTSWLNSEYVNLATMNNVYNAKMNVANFQFLFNMGVRMNLARSKKKGS
||||||||||||||||||||||||||||||||||||||||||||||||||
LAGTSWLNSEYVNLATMNNVYNAKMNVANFQFLFNMGVRMNLARSKKKGS

DHAAQHGIELGLKIPTINTNYYSFMGAELKYRRLYSVYLNYVFAY* 721
||||||||||||||||||||||||||||||||||||||||||||||
DHAAQHGIELGLKIPTINTNYYSFMGAELKYRRLYSVYLNYVFAY* 689
```

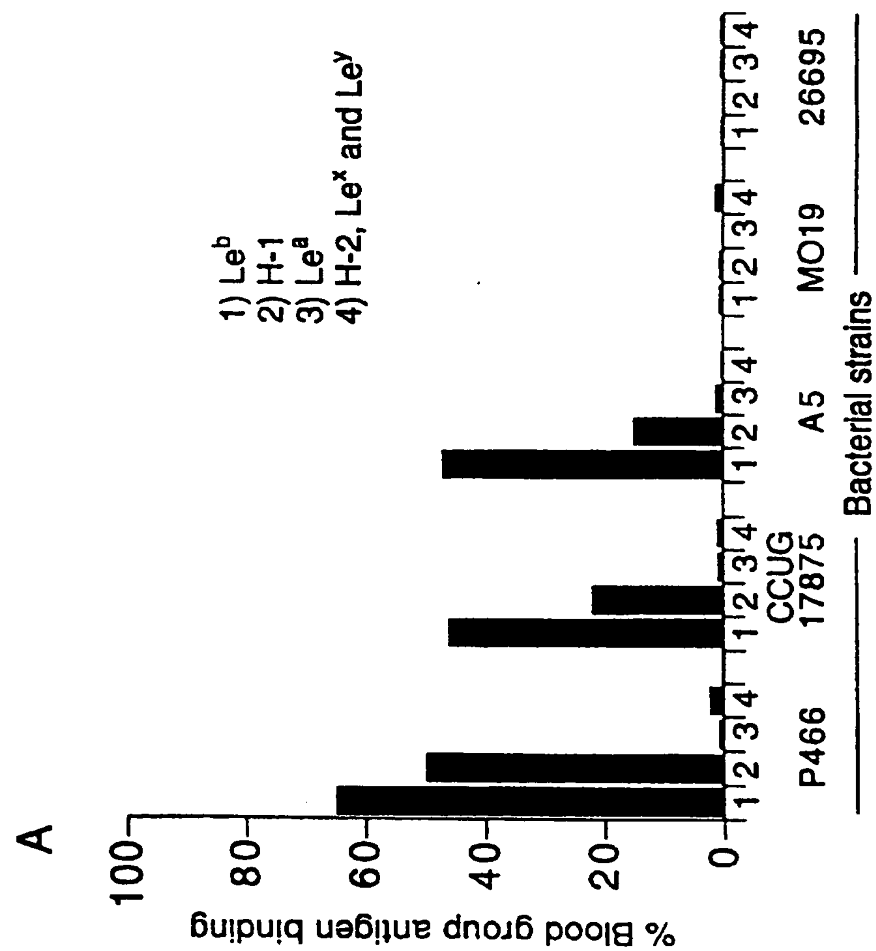
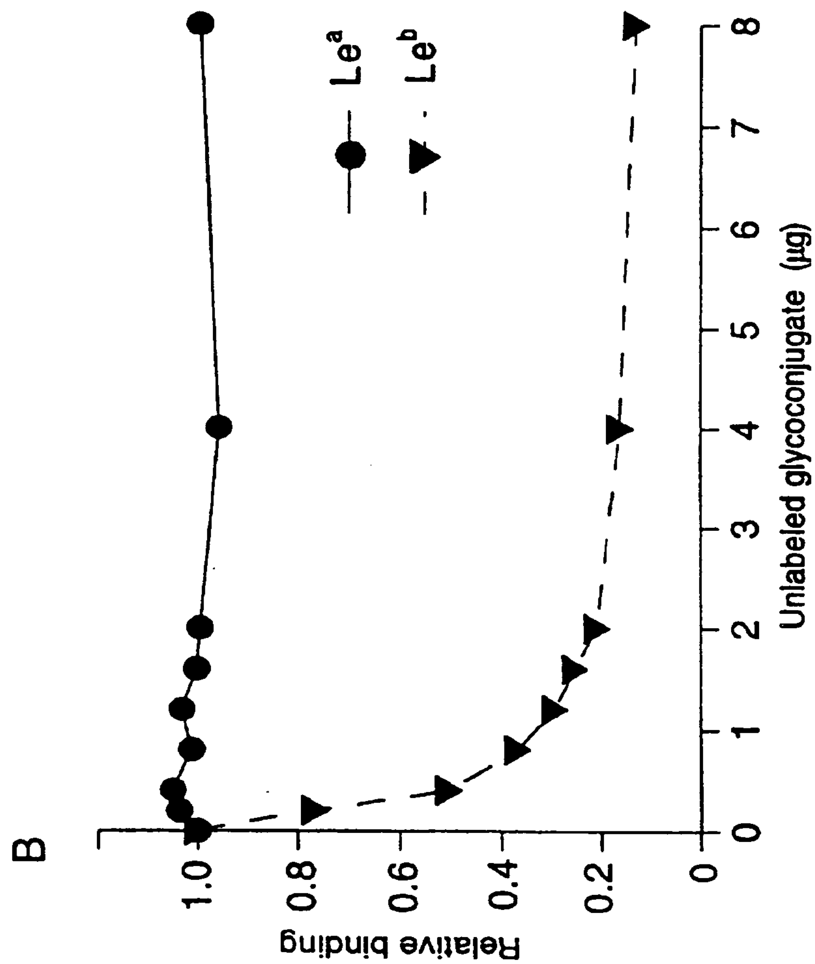

A
% Blood group antigen binding
100
80
60
40
20
0
1) Le^b
2) H-1
3) Le^a
4) H-2, Le^x and Le^y
1 2 3 4
P466
CCUG 17875
A5
MO19
26695
Bacterial strains

Fig. 1A



B
Relative binding
1.0
0.8
0.6
0.4
0.2
0
0
1
2
3
4
5
6
7
8
Unlabeled glycoconjugate (µg)
Le^a
Le^b

Fig. 1B

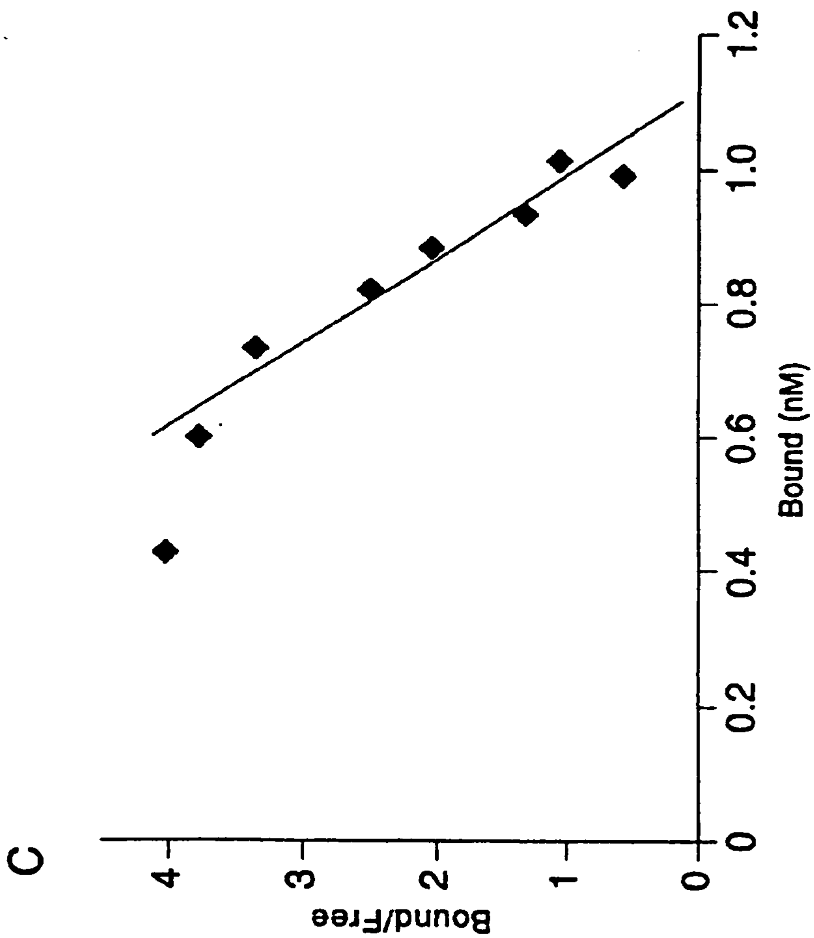


Fig. 1C

Fig. 2

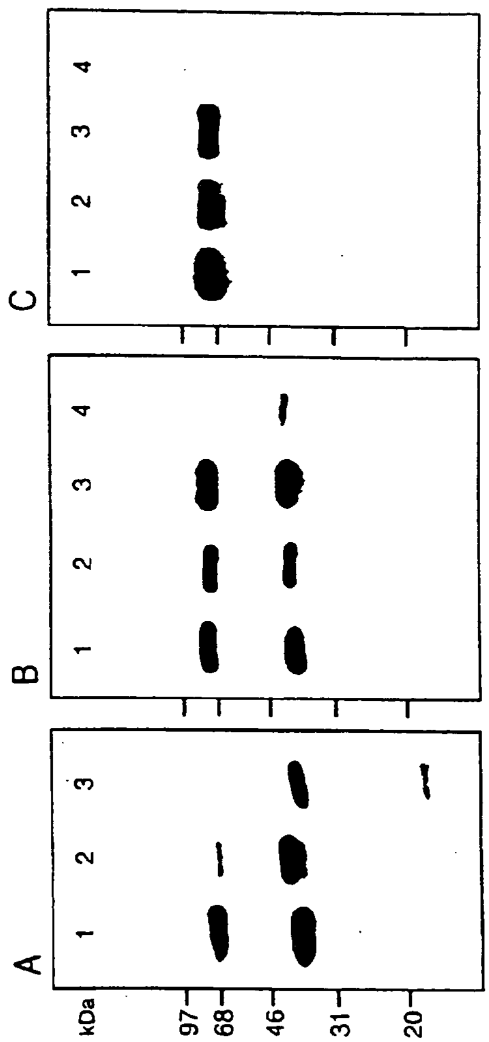


Fig. 3

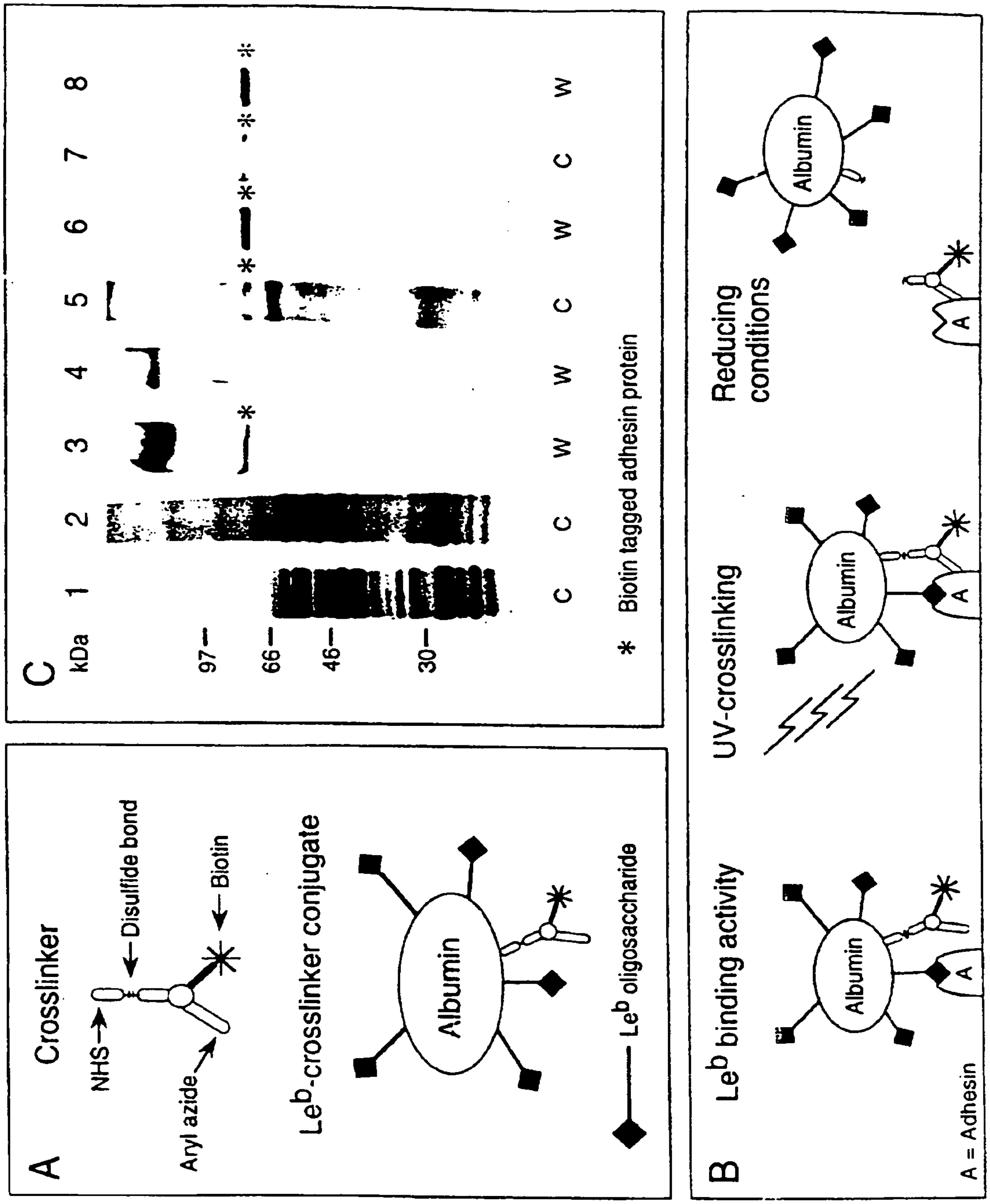

A
Crosslinker
NHS
Disulfide bond
Biotin
Aryl azide
Le^b-crosslinker conjugate
Albumin
Le^b oligosaccharide
B
Le^b binding activity
UV-crosslinking
Reducing conditions
Albumin
A
A = Adhesin
C
kDa
97
66
46
30
1 2 3 4 5 6 7 8
C C W W C W C W
* Biotin tagged adhesin protein

Fig. 4

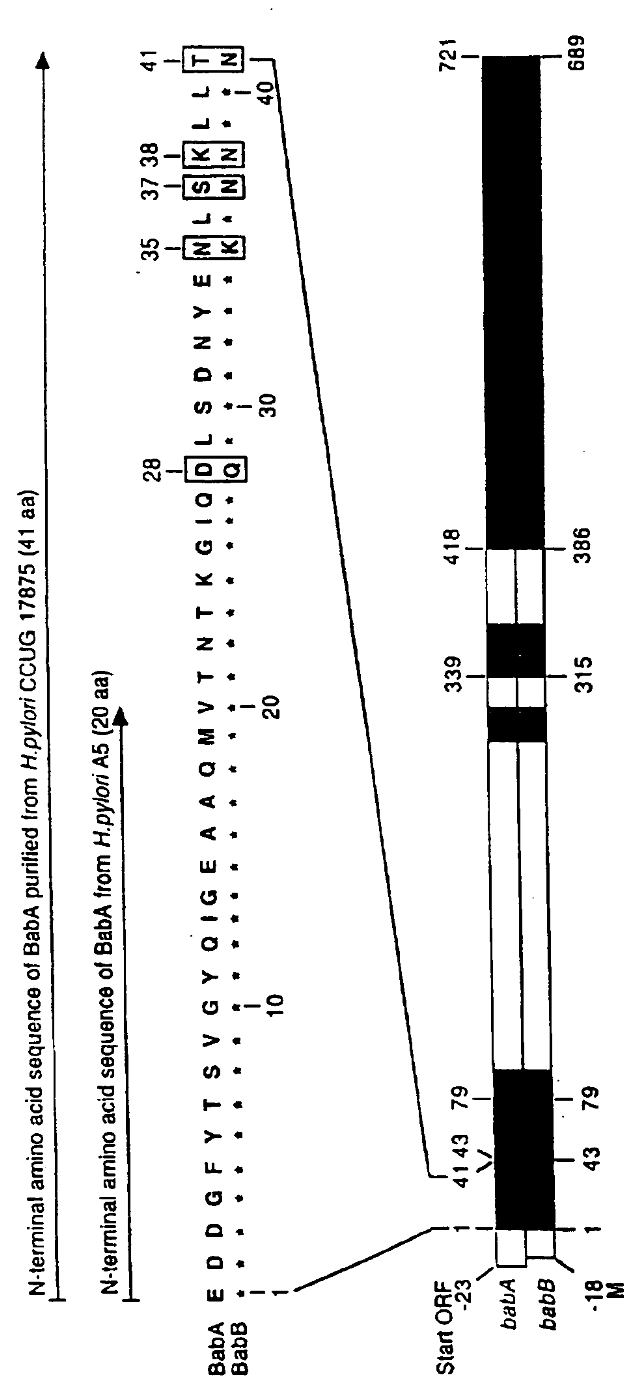

N-terminal amino acid sequence of BabA purified from H.pylori CCUG 17875 (41 aa)
N-terminal amino acid sequence of BabA from H.pylori A5 (20 aa)
BabA
BabB
Start ORF
babA
babB

Fig. 5

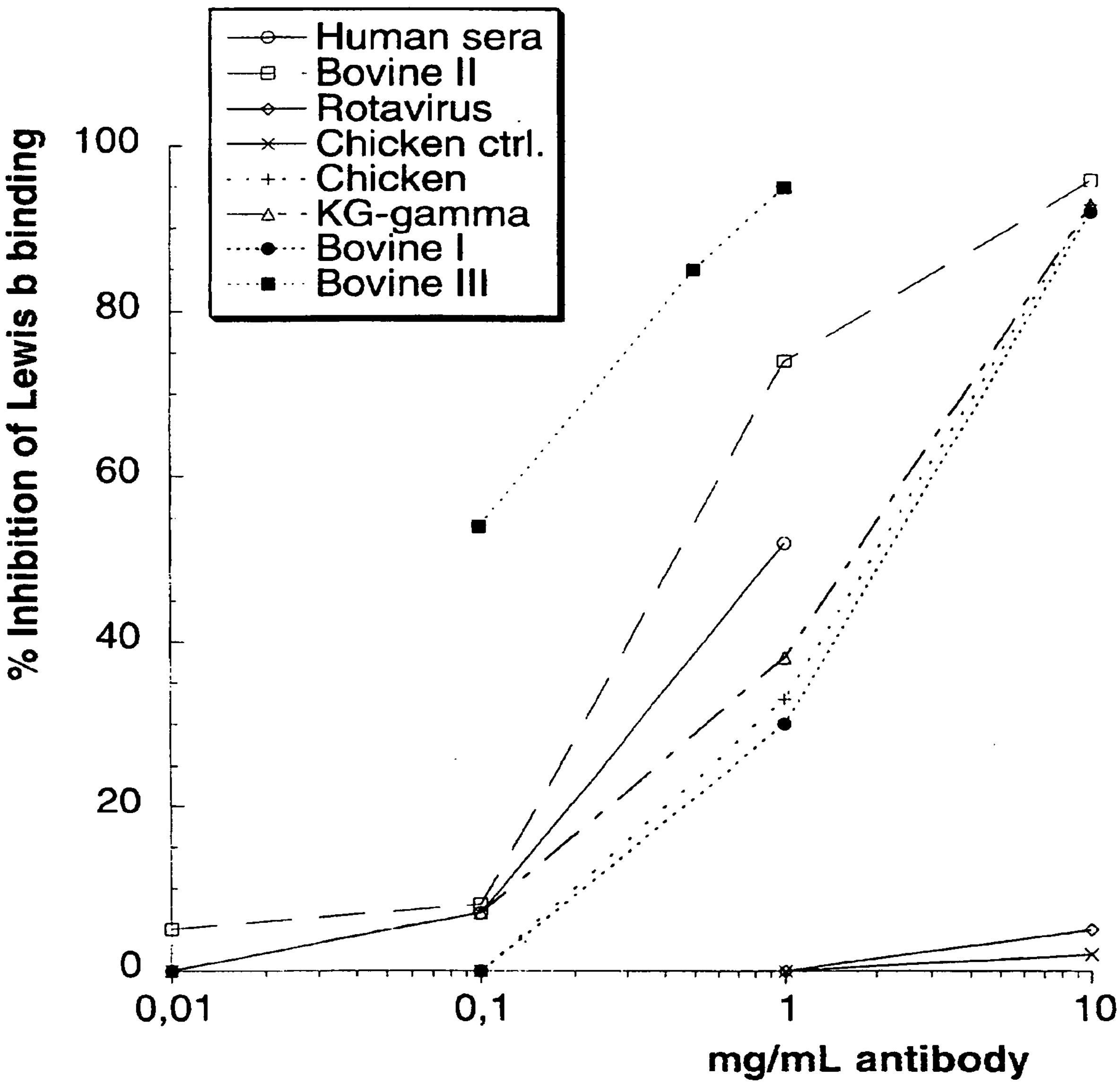

Human sera
Bovine II
Rotavirus
Chicken ctrl.
Chicken
KG-gamma
Bovine I
Bovine III
% Inhibition of Lewis b binding
100
80
60
40
20
0
0,01
0,1
1
10
mg/mL antibody

Fig. 6

Antibody preparations used for Western blot detection of the BabA adhesin and *H. pylori* protein antigens;

Human Sera | Chicken Antibody | Bovine Antibodies I II III II/III

KDa⇓

Lanes: 1 2 3 4 5 6 7 8 9 10 11

Fig. 7

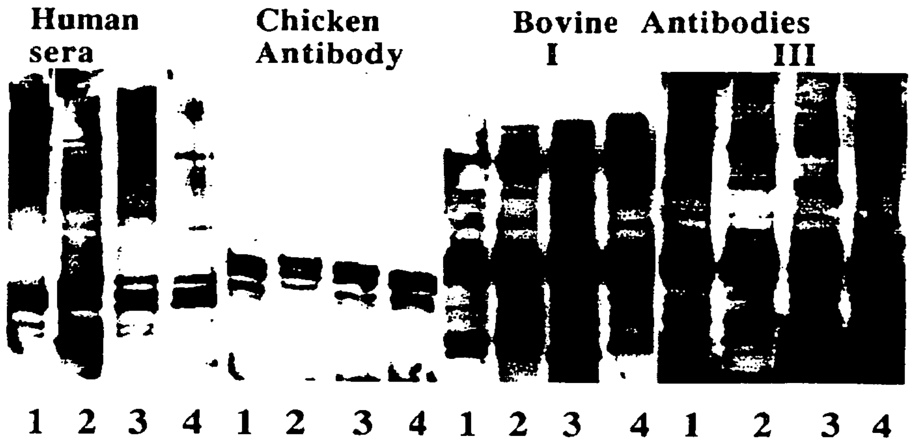

Human sera
Chicken Antibody
Bovine Antibodies
I
III
1 2 3 4 1 2 3 4 1 2 3 4 1 2 3 4

Fig. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 97/01009

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/205, A61K 39/106, C07K 16/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, BIOSIS, DBA, WPI, GENBANK/SWISSPROT/EMBL/DDBJ, REGISTRY FILE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Science, Volume 262, December 1993, Thomas Borén et al, "Attachment of Helicobacter pylori to Human Gastric Epithelium Mediated by Blood Group Antigens" page 1892 --	1-17,24-46, 50
P,A	Dialog Information Services, File 34, Scisearch, Dialog accession no. 15596361, Alkout AM et al: "Isolation of a cell surface component of Helicobacter pylori that binds H type 2, Lewis(a), and Lewis(b) antigens"; & Gastroenterology, 1997, V112, N4 (APR), P1179-1187 --	1-17,24-46, 50

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" ertier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 October 1997	23.10.97
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Patrick Andersson Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 97/01009

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,A	Dialog Information Services, File 34, Scisearch, Dialog accession no. 15313697, Clyne M et al: "Cell-envelope characteristics ofhelicobacter -pylori - their role in adherence to mucosal surfaces and virulence"; & Fems Immunology and Medical Microbiology, 1996, V16, N2 (DEC 1), P141-155	1-17,24-46, 50

--

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 97/01009

Box I **Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 18-23, 47-49
because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1(iv): Methods for treatment of the human or animal body by therapy.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II **Observations where unity of invention is lacking (Continuation of item 2 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
[ ] The additional search fees were accompanied by the applicant's protest.
[ ] No protest accompanied the payment of additional search fees.